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***Nebalia holothurophila*, a New Species of the Leptostraca (Crustacea, Malacostraca) Possibly Commensal with the Sea Cucumber, *Apostichopus parvimensis* (H. L. Clark, 1913), in Southern California**

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Abstract.—A new species of leptostracan of the genus *Nebalia*, *N. holothurophila*, is described from southern California. The new species appears closely associated with the warty sea cucumber, *Apostichopus parvimensis* (H. L. Clark, 1913), although the exact nature of the relationship is unclear. The leptostracans were found in “transfer tubs” after the sea cucumbers were removed from artificial tidepools and placed in fresh seawater within these tubs. The new species differs from other leptostracans in southern California in having a combination of features not shared by any other species.

Leptostracans are small (usually 3 to 12 mm) crustaceans characterized by a large, bivalved carapace that covers nearly all of the thorax and the eight thoracic appendages, a short movable rostrum, and leaf-like appendages. Haney et al. (2007) and Olesen et al. (2014) summarized leptostracan general morphology and ecology. Although they are most often found in and on organic-rich substrates, such as estuarine mudflats and algal mats, they have been reported from a wide range of habitats, including seagrass beds, subtidal detrital mats, marine caves, and even deep-sea hydrothermal vents (Bowman et al. 1985; Haney and Martin 2000; Haney et al. 2007; Olesen et al. 2014). At least one species has been described as living on or in a sponge (Ortiz et al. 2011).

Leptostracans are not a diverse group of crustaceans, with only 10 genera and approximately 60 species described to date. More than half of the species (36) belong to the genus *Nebalia* Leach, 1814, a genus found nearly worldwide. In waters of southern California, relatively few species have been described, although it was noted by Haney et al. (2007) that additional undescribed species exist along this coast, and there has been confusion over the taxonomic status of *Nebalia pugettensis* Clark, 1932, originally described from Puget Sound and at one time believed to be widespread (Haney et al. 2007). Only six species have been reported from California waters: *Nebalia daytoni* Vetter, 1996; *Nebalia gerkenae* Haney and Martin, 2000; *Nebalia kensleyi* Haney and Martin, 2005a; *Nebalia* sp. (described by Haney et al. 2007 as widespread in the Pacific Northwest and previously confused with *N. pugettensis*); *Nebalia hessleri* (Martin, Vetter, and Cash-Clark 1996); and *Nebalia pugettensis* (the identification of which was questioned by Martin et al. (1996) (Haney et al. 2007).

Beginning in 2013, the second author, while volunteering at the Birch Aquarium of the Scripps Institution of Oceanography (La Jolla, California), noticed that when live specimens

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of the warty sea cucumber *Apostichopus parvimensis* (Clark, 1913) were transferred into a new container, sometimes small crustaceans were expelled from the cucumber's cloaca. Some of these crustaceans were eventually collected and sent to the senior author; these specimens and observations form the basis of this report.

Materials and Methods

All specimens were collected by the second author when volunteering at the Birch Aquarium in La Jolla, California. The artificial habitats at the Birch Aquarium that contain the sea cucumbers are best described as outdoor, semi-natural tidepools. These pools are made of concrete, but fresh seawater is flowing through them in pulses, approximately every 60-90 sec. Multicellular algae grow naturally in the pools, though algal biomass is less substantial in the lowest pool (where the leptostracans were found), since it is nibbled away by fishes (zebra perch and/or opaleyes). Other macroinvertebrates in the pools include the urchins *Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus*; the anemones *Anthropleura xanthogrammica* and *A. elegantissima*; the sea stars, *Pisaster giganteus*, *P. ochraceus*, and *Patiria miniata*; and the California spiny lobster, *Panulirus interruptus*. The bottom of the enclosure is covered by a mixture of sand and shell fragments mixed with small fragments of algae. In the lower pools where the leptostracans were found, the bottom is well disturbed by the fishes, making the substrate well aerated (and hence not anoxic). Chunks of krill, squid, and fish are added to the pools twice per week, and clumps of feather boa kelp, *Egregia menziesii*, and/or giant brown kelp, *Macrocystis pyrifera*, are added for the herbivores. As this is a semi-natural tide pool, there is a wide variety of other small invertebrates crawling among the rocks and algae.

Only five specimens have been found to date, four of which were found in containers (plastic "transfer tubs" approximately 25 × 35 × 15 cm) containing only live specimens of the sea cucumber and one of which (the smallest) was taken from algae rather than from one of the tubs. The leptostracans were noticed after water was expelled from the cloaca of the sea cucumbers as a reaction to their placement in a new container. Notably, the leptostracans were not seen being expelled; rather, they were noticed in the tub later, after the sea cucumber had been removed. All specimens were initially stored in isopropanol or commercial (Everclear) alcohol (75.5% ethanol) and shipped to the Natural History Museum of Los Angeles County (NHMLAC), where two specimens were prepared for SEM by cleaning for 10–20 sec in a Branson 1200 ultrasonic cleaner in a weak solution of Branson GP jewelry soap and distilled water. These two specimens were then dehydrated with 100% ethanol. Specimens were placed in solutions of pure ethanol and distilled water in the ratios 2:1, 1:1, 1:2, and finally into 100% ethanol (20 min per treatment). Once dehydrated, specimens were transferred through a series of ethanol and hexamethyldisilazane (HMDS) solutions in the ratios 2:1, 1:1, 1:2 and finally into 100% HMDS (20 minutes per treatment). Specimens then were transferred from the final 100% HMDS to fresh HMDS and allowed to evaporate overnight; they were then mounted on carbon conductive tabs and coated with gold-palladium using an Emitech K550x sputter coater (Quorum Technologies, LTD, Kent, UK) and imaged using a Hitachi S-3000N variable pressure SEM (Hitachi, Troy, MI) at the NHMLAC. The remaining specimens were maintained in 70% ethanol for permanent storage.

Drawings of the entire animal were made using a Wild M5APO dissecting microscope with a drawing tube attached; drawings of the limbs and mouthparts were made with a Nikon Labophot compound microscope equipped with a drawing attachment.

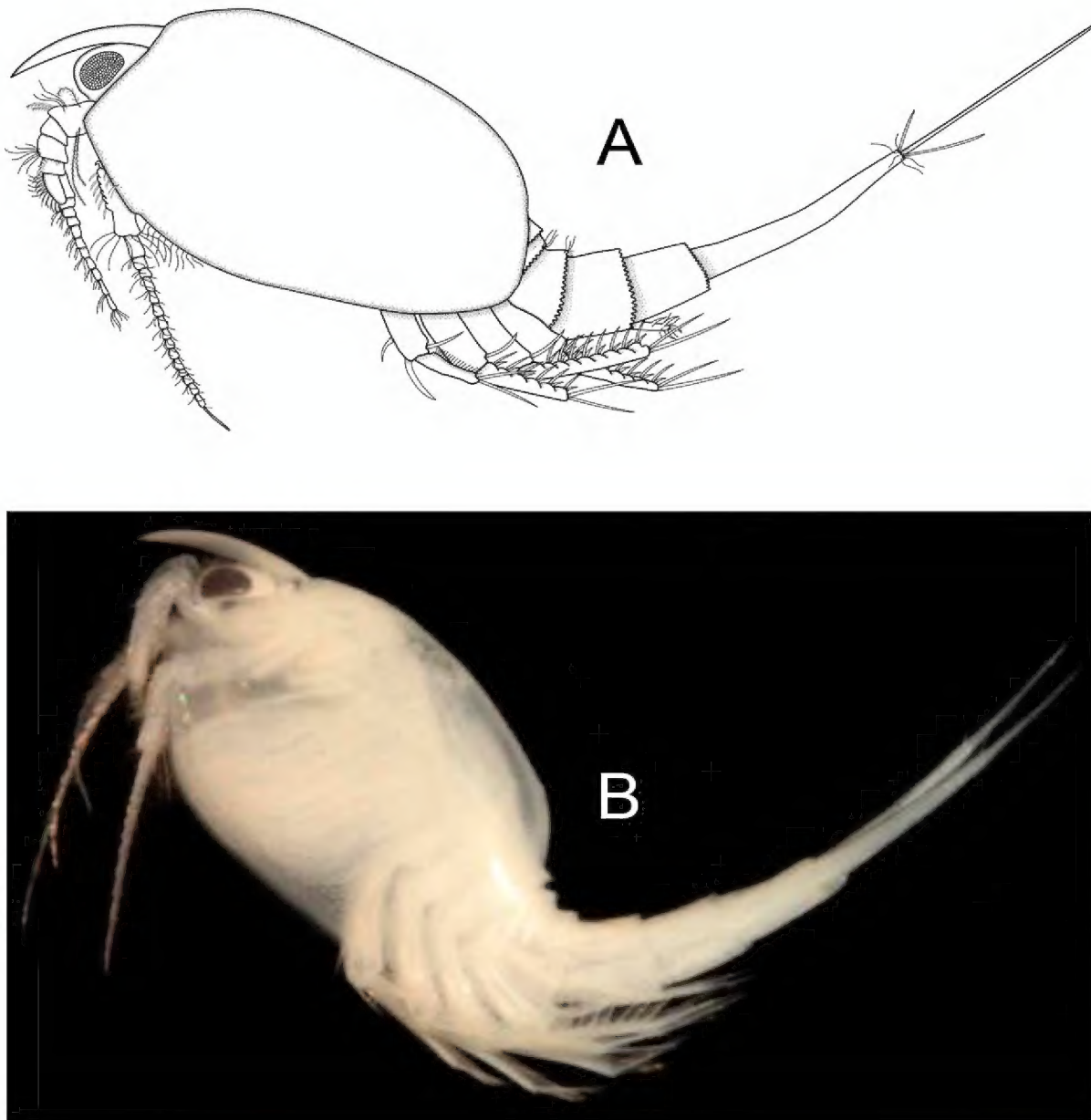


Fig. 1. *Nebalia holothurophila* n. sp., holotype female LACM:DISCO:28304, lateral (left) side. A, illustration based on freshly preserved specimen. B, color photograph of same specimen.

Results

Description (Holotype)

Whole animal length 3.33 mm. Carapace (Fig. 1A, B) broad, ovate, gently rounded but with slight angle at anteroventral border, approximately level with base of the antennule, carapace length in lateral aspect 1.9 mm long, height 1.4 mm, and 0.875 mm deep (Fig. 1A).

Rostrum (Figs. 1A; 2A, F) long, length approximately $2.5 \times$ width (length 1.10 mm; width 0.44 mm), acute in lateral view but anteriorly broadly rounded in dorsal or ventral view, slightly downturned, extending to approximately twice the length of the eye. Subrostral keel (Fig. 2A) rectangular.

Eyes (Figs. 1A, B; 2B, F; 5A) bluntly rounded distally, with anteroventral angle more acute than anterodorsal angle. Ocular plate above eye (Fig. 2B) acute, extending to approximately half the length of the eyestalk.

Antennule and antenna.—Antennular peduncle (Fig. 1C) composed of three articles, the first longer than the second and third combined, third (Fig. 5C) terminating in two stout, smooth spines (Fig. 5C, arrow); antennular scale (Figs. 2D; 5B, D) broadly rounded distally, with row of 15-18 short setae opposite a row of 10-12 longer setae lining anterior margin. Flagellum with 10 short articles.

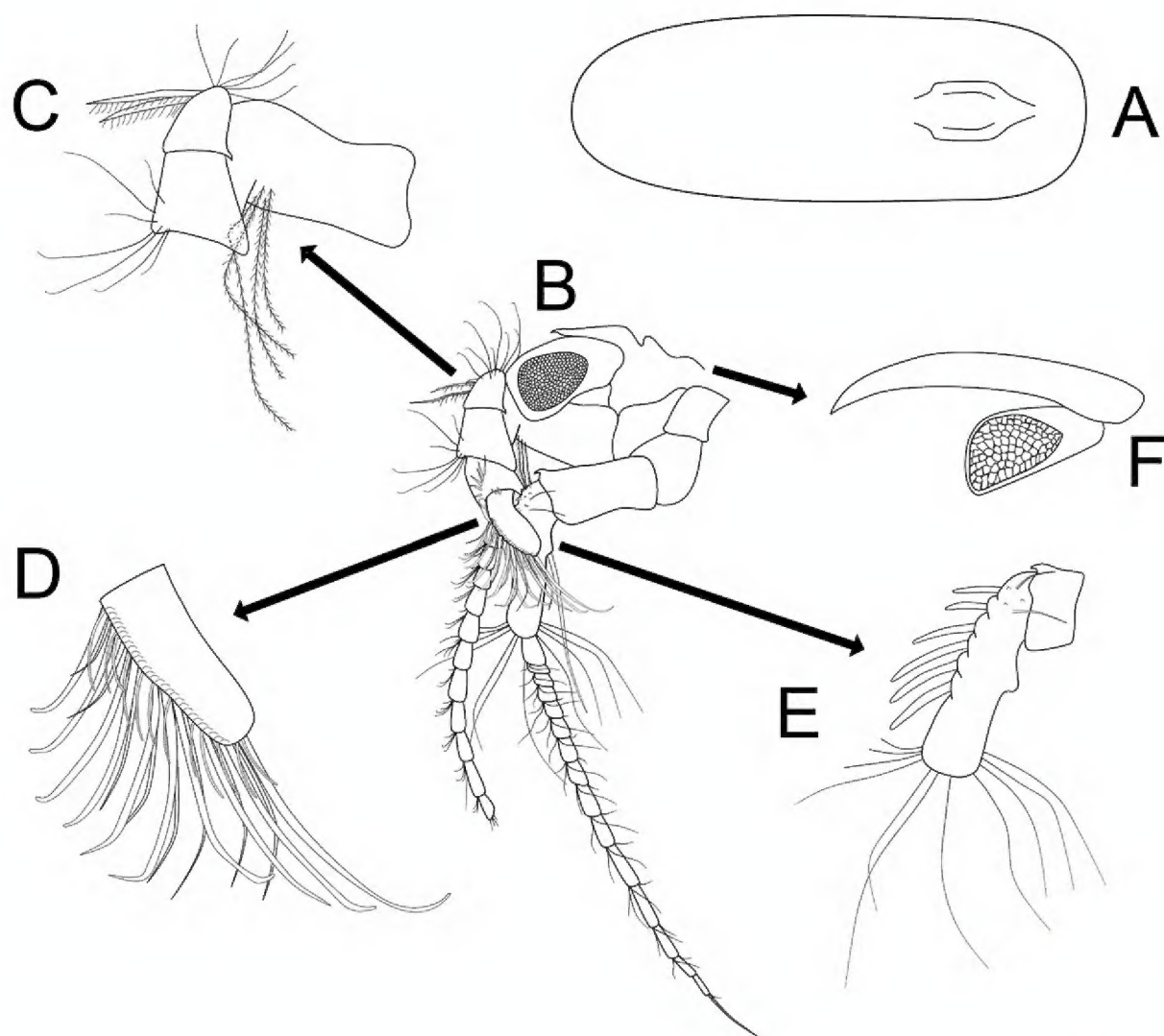


Fig. 2. *Nebalia holothurophila* n. sp., paratype female LACM:DISCO:28305. A, rostrum in ventral view. B, eye, antennule, and antenna in lateral (left side) view. C, proximal three articles of antennular peduncle. D, antennular scale. E, distal (fourth) article of antennal protopod. F, rostrum and eye, lateral (left) view.

Antenna (Figs. 2B, E; 3A) with three articles on peduncle, first unarmed, second with a short distolateral spine, third with 5 to 6 progressively larger spines along the anterior proximal border and several terminal setae; flagellum with 15 articles.

Mandible. Not examined.

Maxillule (Figs. 2B, E; 4B, C) with basal endite wide and extremely short, followed by broadly rounded endite lined with approximately 10 terminally spatulate spines along medial margin; palp bearing approximately 15 distally hooked setae along medial margin.

Maxilla (Fig. 4A) with four basal endites, setation of which is 8, 7, 13-16, and 4, respectively; endopod of two segments, proximal article longer than distal, exopod composed of single article; endopod and exopod bearing long, plumose setae.

Pleonites (Figs. 1A, 5F) with crenulate borders bearing rounded teeth. Pleopod 1 (Fig. 5E) with up to 30 stout, distally serrate spines; pleopod 5 (Fig. 3B) with 4 to 5 long, smooth, terminal spines.

Anal somite, anal plates, uropods.—As per the genus *Nebalia* (see Haney et al. 2007).

Type Material

Holotype (here designated): ♀ (3.33 mm) undissected and used for full body illustrations: California, San Diego County, La Jolla, Birch, 32.8658°N 117.2507°W, preserved in 70% ethanol, 10 Nov 2014. Collector Jerry Jacobs, LACM:DISCO:28304.

Paratype material: All specimens have the same locality data as holotype. LACM:DISCO:28305 was dissected for illustration of appendages and retained in 70% ethanol. LACM:DISCO:28306 1

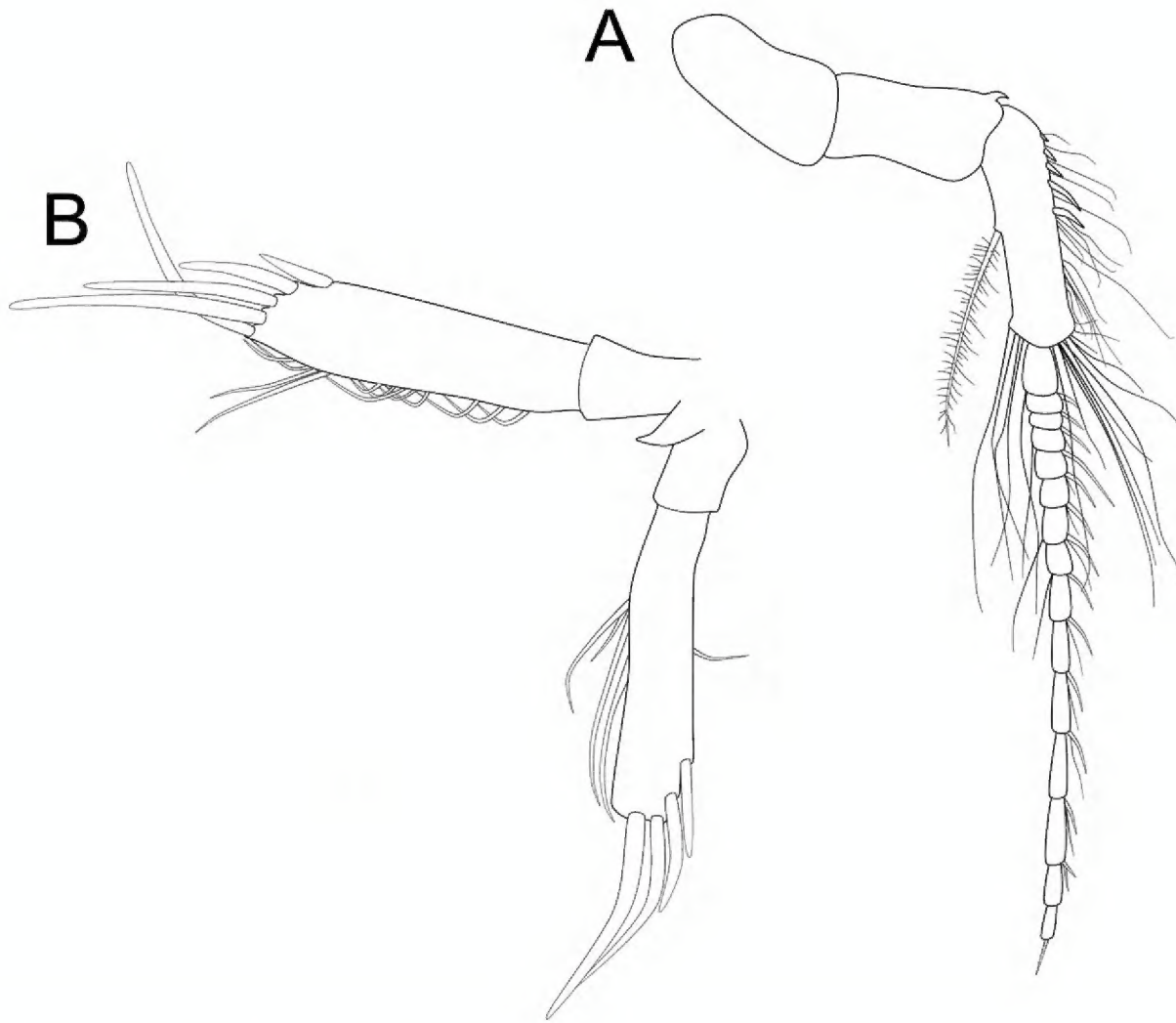


Fig. 3. *Nebalia holothurophila* n. sp., paratype female LACM:DISCO:28305. A, antenna, lateral view. B, pleopod 5, ventral view.

juvenile specimen was fixed in isopropanol of unknown concentration, preserved in 70% ethanol. 2 specimens (LACM:DISCO:28307 and LACM:DISCO:28308) were prepared for SEM and are retained as dried specimens that have been sputter coated in gold-palladium alloy although they have been badly damaged by the drying process.

Etymology

The species is named in recognition of the possible association with the warty sea cucumber, *A. parvimensis*.

Discussion

Of the known species of leptostracans along the southern California coast, the new species is immediately distinguishable from *Nebalia daytoni* by the truncate eyestalk of *N. daytoni* (Vetter 1996; Haney et al. 2007). *Nebalia hessleri* is a large species (averaging more than 6.0 mm body length) and is characterized by having acute (rather than rounded) teeth along the posterior borders of the abdominal somites (Martin et al. 1996), although variation exists both ontogenetically and depending on where along the posterior border of the somites these teeth are examined. Juvenile specimens of *N. hessleri* have more acute teeth along these borders. The other two described California species, *Nebalia gerkenae* and *Nebalia kensleyi*, are distinct geographically, with both reported only north of Monterey Bay. *Nebalia gerkenae* has been found to date only in Bennet Slough, Monterey Bay, California. *Nebalia kensleyi* has been reported only north of Monterey Bay (Haney and Martin 2005b). Additionally, there are slight but significant

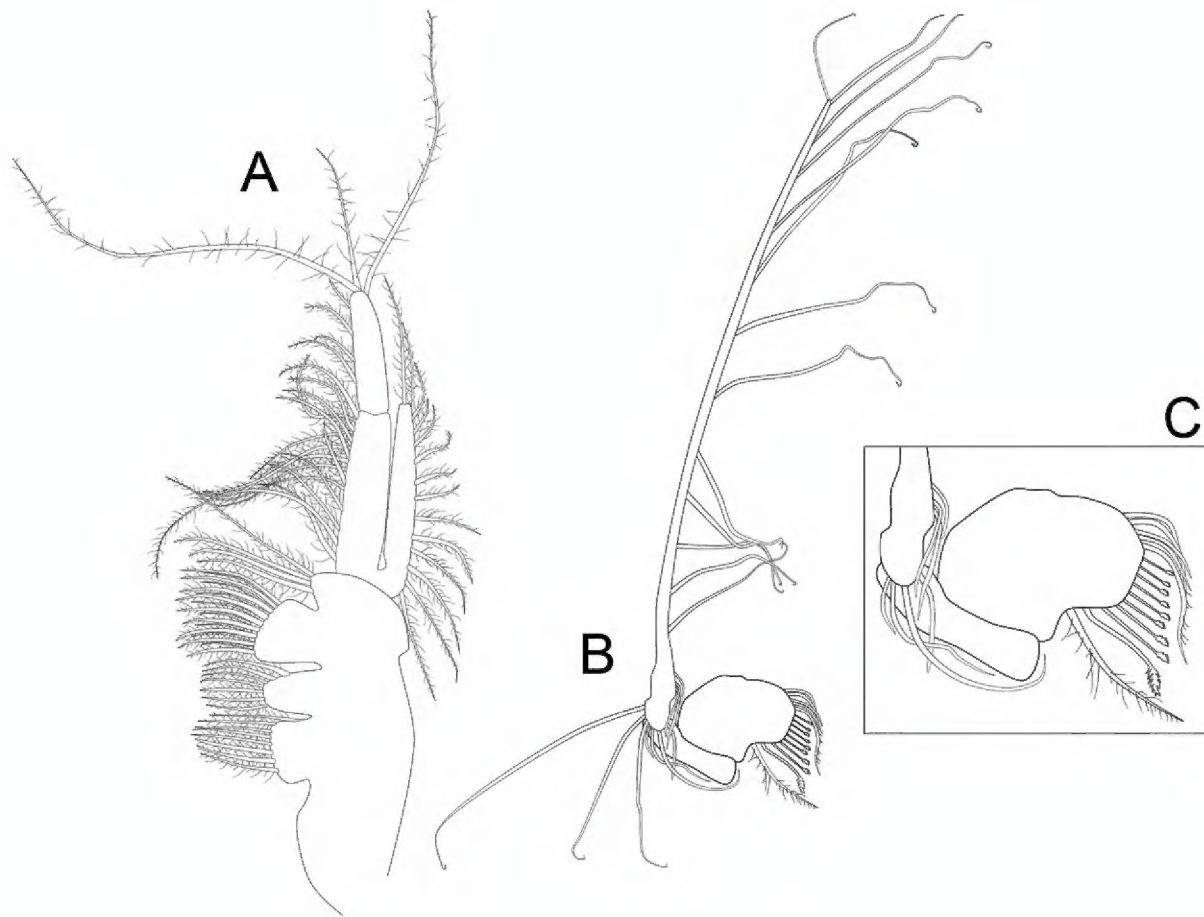


Fig. 4. *Nebalia holothurophila* n. sp., paratype female LACM:DISCO:28305. A, maxilla. B, maxillule. C, higher magnification of basal endite of maxillule.

morphological differences between these more northern species and the new species described herein. In *N. gerkenae*, the teeth of the posterior border of the fourth pleonite taper to a more acute point, rather than being gently rounded as in *N. holothurophila* n. sp. The dorso-distal border of the first and second articles of the antennal protopod bear a stout recurved spine (see Haney and Martin 2000, fig. 3b), whereas this spine is absent on the first such article of the new species. In *N. kensleyi*, there are 30 to 37 dentate spines along the exopod of pleopod 1, whereas the new species has 30 or fewer.

The identity, characteristics, and geographic boundaries of the more northern species that traditionally was known as *N. pugettensis* remain in some doubt until such time that a neotype can be designated and described (Haney et al. 2007; Haney and Martin 2000; Martin et al. 1996). Analyses of nucleotide sequence data from cytochrome oxidase subunit 1 (COI) of leptostracans along the West Coast of North America show that clades from the type locality of *N. pugettensis* indeed differ from those in waters of southern California (Haney, unpublished).

Warty sea cucumbers, *A. parvimensis*, are known from the intertidal and to a depth of about 60 m from Monterey Bay to southern Baja California, although they are more common from southern California to Baja California, usually inhabiting low-energy environments and associated with rock or other hard substrate (Hamel and Mercier 2008). They are harvested as part of a small sea cucumber fishery in southern California and Mexico (Chávez et al. 2011). Warty sea cucumbers are epibenthic deposit feeders (Muscat 1982; Yingst 1982). Like many holothuroids, the species when threatened can eject internal organs (i.e., autoeviscerate) and grow new ones. It is not known how or if the leptostracans avoid this phenomenon.

Various crustacean taxa are known to live in association with holothuroides. For instance, caprellidan amphipods and copepods have been collected from the surface of sea cucumbers (Lindsay and Takeuchi 2008; Mahatma et al. 2008). Other species can be found living within

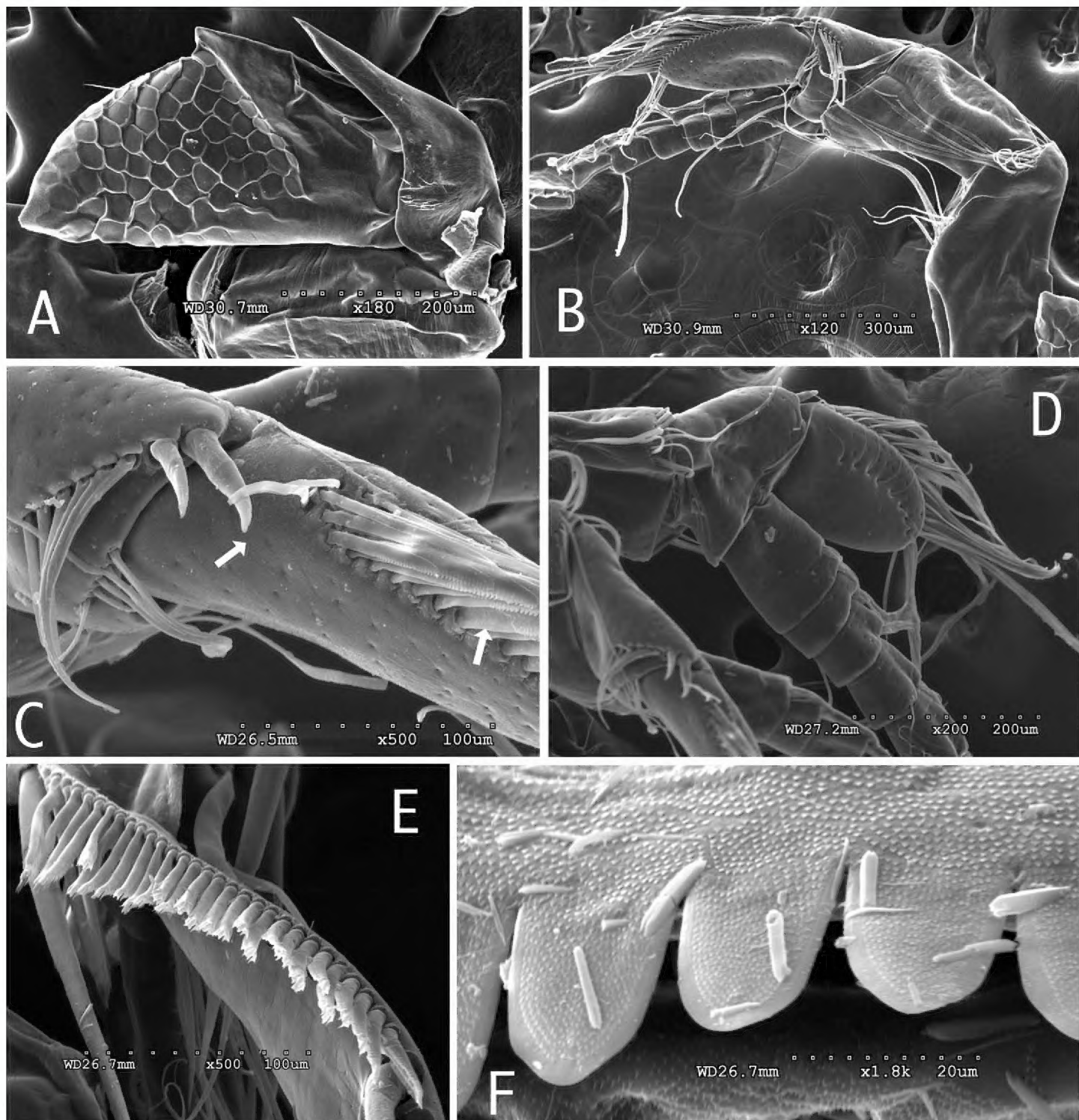


Fig. 5. *Nebalia holothurophila* n. sp., paratypes LACM:DISCO:28307 and LACM:DISCO:28308 (damaged in SEM preparation). A, eye and ocular acicle. B, antennule and antennular scale. C, antennule, distal end of fourth and basal end of fifth antennular articles. D, antennular scale and proximal 5 articles of flagellum. E, row of dentate setae (28) on posterior border of exopod of pleopod 1. F, rounded teeth of posterior border of pleonite 4.

the body of the holothuroid, with associations that range from commensal to parasitic. Records include some portunid crabs of the genus *Lissocarcinus* Adams and White, 1849; as well as xanthoid crabs, copepods, and tanaids (Vandenspiegel et al. 1992; Alvaro et al. 2011; Avdeev 2017). Nineteen species of pea crabs have been recorded from the intestine or respiratory tree of sea cucumbers (de Gier and Becker 2020); these records include the species *Opisthopus transversus* Rathbun, 1894, from within the sea cucumber under study herein, *A. parvimensis*. Although the possibility exists that the new leptostracan species is free-living, or associated with one of the other species in the artificial tidepools, four of the five specimens were found only when the sea cucumbers were isolated in smaller plastic tubs, indicating the strong possibility that these leptostracans are found in, or on, the sea cucumbers. It is possible that the leptostracans are living within the respiratory tree of *Apostichopus*. Collectors of

warty sea cucumbers in the future are encouraged to isolate the live animals for a short period of time (e.g., 24 hr) to see if additional specimens of the leptostracans can be procured.

Acknowledgements

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Phylogeography of the Pacific Sardine, *Sardinops sagax*, across its Northeastern Pacific Range

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Abstract.—The Pacific sardine, (*Sardinops sagax*), is a small, coastal pelagic species in the family *Clupeidae*. Sardine are ecologically important forage for many animals, and have historically supported a large commercial fishery. To expand on previous evolutionary genetic studies of population structure and to test if population structure is present in Pacific sardine was reflective of long-term processes, 434 individuals were examined ranging from Vancouver Island, British Columbia to Bahía Magdalena, Baja California, and from the Gulf of California. A 1062 bp fragment of the cytochrome *b* gene yielded small but significant fixation estimates of Φ_{ST} (0.01136, $p = 0.032$). Concordantly low fixation was observed for two Φ_{CT} groupings (0.00435, $p = 0.128$ and 0.00923, $p = 0.021$). These data support the null hypothesis of an absence of genetic structure in the Pacific sardine.

Analysis of population structure is important to the study of species and to the field of evolutionary biology. Molecular analysis of populations in relation to hypothesized barriers to gene flow can elucidate questions of population structure. Biogeographic boundaries occur between areas with unique assemblages of fauna, and at times, they may align with intra-species phylogeographic breaks, indicating concordance of a biogeographic boundary with a barrier to gene flow (Avice 2000). A question that remains is how frequently this concordance occurs in oceans where barriers to dispersal are less obvious than in terrestrial systems.

The marine ecosystems of the northeastern Pacific Ocean, ranging from the warm waters of the Gulf of California north to the colder waters of Alaska are home to a diverse assemblage of marine organisms. This region is represented by several distinct assemblages of marine fishes representing multiple biogeographic or faunal provinces. Traditionally, these provinces have been described as the Aleutian province, extending from Nunivak Island in Alaska through the Dixon Entrance (U.S.-Canada border), the Oregonian province from the Dixon Entrance southward to Point Conception (Briggs and Bowen 2012 consider the southernmost boundary to be the Los Angeles region), the Californian Province extending from Point Conception (Briggs and Bowen 2012 consider the northernmost boundary to be the Los Angeles region) to Bahía Magdalena in Mexico, and the Cortez Province from Bahía Magdalena around the southern end of the Baja California Peninsula into the Gulf of California (Briggs 1974; Horn and Allen 1978; Boschi 2000; Hastings 2000; Briggs and Bowen 2012).

Arguably the most well-studied of the traditional biogeographic breaks within the range of the Pacific sardine is Point Conception, which has long been recognized as a biogeographic

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break separating the cold Oregonian and temperate Californian fish assemblages (Briggs 1974; Horn and Allen 1978; Boschi 2000). The waters surrounding this prominent headland are characterized by complex oceanographic patterns resulting from the convergence of the warmer surface waters of the Southern California Bight and the colder surface waters north of the point (Wang 1997; Hohenlohe 2004). Historically, these oceanographic differences have been considered to act as a barrier to dispersal of adult and larval organisms. Briggs and Bowen (2012), however, argue that this area is more of a transition zone than a boundary. With this in mind, it is interesting to ask if the same processes acting on species distributions in this region also act on an intraspecific level. That is, is Pt. Conception a barrier to gene flow (i.e., a phylogeographic break driving population separation), or does it act more as a diffusive membrane (i.e., allowing sufficient gene flow to prevent evolutionary divergence)? While some studies have shown the former, others have shown the latter (Burton 1998; Bernardi 2000; Dawson et al. 2001; Craig et al. 2011).

The geographic range of the Pacific sardine also includes other hypothesized barriers to dispersal. On the western coast of the Baja California Peninsula, the northern end of the Cortez province is marked by a strong temperature gradient at Bahía Magdalena (Palacios-Salgado et al. 2012). The biogeographic boundary at Punta Eugenia has also been implicated as a phylogeographic boundary zone for marine organisms, with many examples of nearshore species in the Eastern Pacific showing reciprocal monophyly and strong genetic divergence among disjunct populations in the Gulf of California (Terry et al. 2000; Bernardi et al. 2003). The Gulf of California is considered as part of the Cortez faunal province (Hastings 2000; Briggs and Bowen 2012; Palacios-Salgado et al. 2012), and, as with the boundary of the Oregonian and Californian provinces, some discrepancy exists on the exact delineation of its endpoints (Briggs and Bowen 2012).

In marine fishes, the primary driver of gene flow is dispersal, either as adult movement or egg and larval drift between regions or groups. Hypothesized barriers to dispersal such as Point Conception and Punta Eugenia may be more relevant to benthic, estuarine, or live-bearing fish with lower dispersal ability by acting as more effective barriers to gene flow over long time scales (Bernardi 2000; Bernardi and Talley 2000; Buonaccorsi et al. 2005; Johansson et al. 2008; Craig et al. 2011). The phylogeography of demersal fishes on the west coast of North America is broadly represented in the literature (e.g., Bernardi 2000; Bernardi and Talley 2000; Terry et al. 2000; Dawson et al. 2001; Cope 2004; Craig et al. 2011), yet studies on pelagic species are less so. Additional investigation of population genetic data for pelagic species in the northeastern Pacific Ocean may facilitate an understanding of biogeographical patterns and gene flow in marine fishes.

Pacific sardine (*Sardinops sagax*) are clupeid fish that are at times the dominant forage fish in the California Current (Preti et al. 2001; Emmett et al. 2005; Lo et al. 2011; McClatchie et al. 2017). Pacific sardine spawn at various intensities year-round through a wide range of temperatures often with peaks in activity during the spring (Ahlstrom 1954; Ahlstrom 1959; Ahlstrom 1965; Hernandez-Vazquez 1994; McFarlane et al. 2005). Recent examination of CalCOFI and IMECOCAL data across Alta and Baja California from 1963-2015 showed that sardine larvae were present across all seasons but were typically at their lowest abundance during winter (Tran 2023). Sardine also spawn in the Gulf of California during the cooler winter months (Hammann et al. 1998; Aceves-Medina et al. 2009). Sardine eggs are planktonic (Zweifel and Lasker 1976), and after hatching larvae absorb the yolk as they develop (Lasker 1965) and may take between 5 and 8 d to deplete the yolk sack depending on the oceanic conditions (Politikos et al. 2018). In the California Current Ecosystem (southern Canada to

southern Baja California), the range for suitable egg and larval survival is thought to be 13–16 °C (Lasker 1964; Agostini et al. 2007; Dorval et al. 2019). Juvenile sardines are strict filter feeders (Arthur 1976), but as adults they also exhibit particle feeding (Emmett et al. 2005). Tagging studies have shown that at least a portion of Pacific sardine biomass migrates extensively each year, moving northward in the spring and returning to warmer southern waters in the fall (Clark and Janssen 1945; Lo et al. 2011; Dorval et al. 2019). These studies have shown that older, larger fish tend to migrate farther than smaller, younger fish (Clark and Janssen 1945). Parasite data have also suggested that not all individuals make an annual migration (Baldwin 2010; Baldwin et al. 2012; Jacobson et al. 2019).

Previous genetic studies have given no indication of deep genetic structure in Pacific sardine (Hedgecock et al. 1989; Lecomte et al. 2004; García-Rodríguez et al. 2011). However, these studies analyzed comparatively few individuals and/or used methods that were common practice at the time of publication but are no longer widely used (e.g., allozymes). In an effort to further our understanding of the phylogeography of Pacific sardine and to understand the effect of hypothesized barriers to gene flow over evolutionary time scales in a coastal pelagic species, this study expands on previous research by including a larger sample size and a more fine-scale/broader geographic sampling range. Given the ecology and high motility of the Pacific sardine, it was considered unlikely that hypothesized barriers to gene flow would act as long-term barriers to gene flow in Pacific sardine along their northeastern Pacific range.

Materials and Methods

Tissue samples from the USA were sourced from the archival collection at the Southwest Fisheries Science Center in La Jolla, California, that were collected during surveys of coastal pelagic species aboard NOAA research vessels (Cutter and Demer 2008). Samples from Mexico were collected through collaborative port/fisheries sampling programs. All samples consisted of muscle tissue or fin clips stored in 100% ethanol at ambient temperature. Twelve sampling locations were selected. These included Vancouver Island (Canada), Washington State (USA), northern, central, and southern Oregon (USA), Crescent City (USA), Pescadero (USA), Monterey (USA), Morro Bay (USA), Point Conception (USA), San Diego (USA), Ensenada (Mexico), Isla Cedros (Mexico), Bahía Magdalena (Mexico), El Desemboque (Mexico), and Guaymas (Mexico) (Fig. 1). All samples were collected from 2002–2006. Total genomic DNA extraction was performed using the Qiagen DNeasy 96 Blood and Tissue Kit following manufacturer's protocol with the exception of the final elution step which was performed using 50 µl of DI water.

A 1500 bp portion of the Cytochrome *b* gene was amplified in two ~750 bp fragments using the polymerase chain reaction (PCR). Primers were designed in house using reference sequences available from GenBank (Table 1). PCR reactions consisted of 1 µl 10X buffer (670 mM Tris, 166 mM (NH₄)₂SO₄, 20 mM MgCl₂, 100 mM 2-mercaptoethanol, pH = 8.8), 1 µl dNTPs at 2 mM, 0.25 µl BSA at 20 mg/ml, 0.5 µl forward and reverse primer at 10 µM, 5.7 µl MilliQ water, 0.05 µl Taq 5 U/ul, and 1 µl DNA at 10–100 ng/µl. Thermal cycling parameters were: 94 °C for 2:00 min, 35 cycles of 94 °C for 30 sec, 47 °C for 30 sec, 72 °C for 1 min 15 sec, with a final extension at 72 °C for 5 min. PCR products were visualized by gel electrophoresis using 2% agarose gel with ethidium bromide to check for successful amplification. PCR products were purified by adding 6 µl of ExoSap solution (Exonuclease I at 3.33 U/µl and Shrimp Alkaline Phosphatase at 0.66 U/µl) and heating to 37 °C for 15 min followed by 80 °C for 15 min. Sanger sequencing was performed using BigDye Terminator v3.1 using

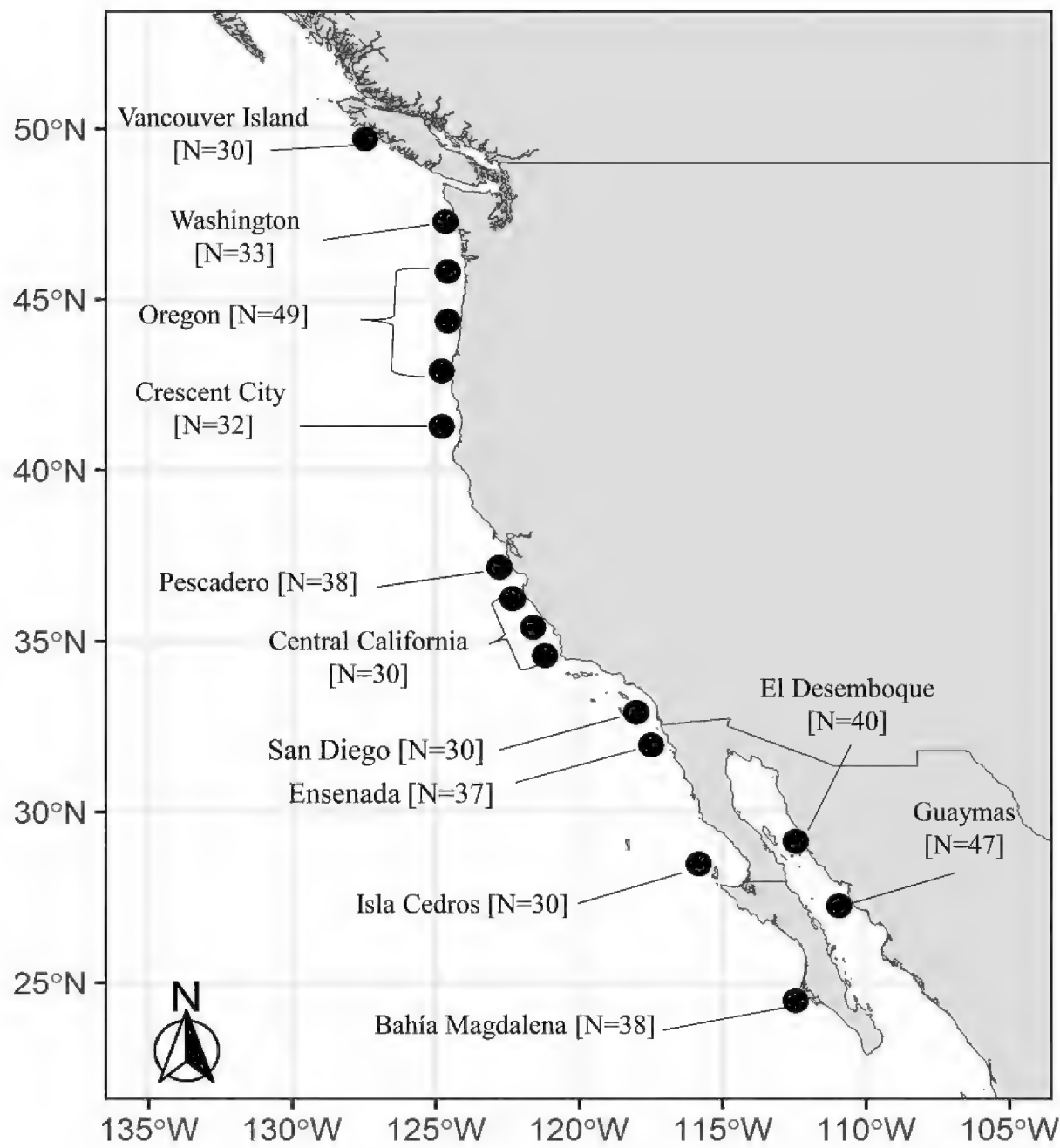


Fig. 1. Sampling locations for 434 total Pacific sardine.

the following master mix: 2 µl of 5x Sequencing Buffer, 1 µl BigDye Terminator v3.1 Ready Reaction Mix, 0.3 µl primer at 10 µM, 5.2 µl of MilliQ water, and 3.5 ul PCR product. Sequence reactions were cleaned using ethanol/salt precipitation and centrifugation. 86 µl of a high salt precipitation solution (3 ml of 3 M NaOAc pH 5.2 ± 0.1, 22.5 ml of MilliQ H₂O, 62.5 ml of 100% EtOH) was added and samples were centrifuged for 20 min at 3700 rpm. Samples were drained and washed with 150 µl of 70% ethanol twice and resuspended in 10 µl of HiDi formamide. Samples were then denatured at 95 °C for five min, and sequenced on an ABI3730 Genetic Analyzer.

Table 1. Primers for each fragment of cytochrome *b*, created by Integrated DNA Technologies from Genbank sequences.

Primer	Sequence
Set 2 Forward	5' GGAATCTAACCGAGACTAATG 3'
Set 2 Reverse	5' GGAGGTTAGGTGAGAAGA 3'
Set 4 Forward	5' GCTATGTTCTGCCTTGAG 3'
Set 4 Reverse	5' CTCCGGTCTTCGGATTA 3'

Table 2. Estimates of Tau (T), Theta naught (Θ_0), Theta one (Θ_1) used to calculate coalescence time computed in *Arlequin* v3.5 (Excoffier and Lischer 2010).

Parameter	Estimate
T	5.000
Θ_0	3.600
Θ_1	7254.954

Contiguous consensus sequences were concatenated from forward and reverse strands, edited, aligned, and trimmed to 1062 bp using Sequencher v5.0 (Sequencher sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA). Those not reaching this size threshold were eliminated from further analysis. After size selection, 434 individuals were retained. Aligned sequences were analyzed for population structure using *Arlequin* v3.5. (Excoffier and Lischer 2010), and the R package *strataG* (Archer et al. 2016). All analyses done in R were performed using R Statistical Software v4.2.2 (R Core Team 2022). Haplotype and nucleotide diversities, as well as neutrality tests for Tajima’s D and Fu’s F_S were calculated in *Arlequin* by sampling site, and all tests were run with 10,000 permutations. A comparison of pairwise differences was computed in *Arlequin* with 1000 permutations with the significance level set to 0.05. Fixation indices (Φ_{ST} and Φ_{CT}) were calculated using *strataG* v2.5.01. Φ statistics were calculated for samples grouped into a Pacific group and a Gulf of California (GOC) group, with Bahia Magdalena tested in each group. Φ_{ST} was also tested for all samples pooled into one single group. Overall haplotype and nucleotide diversities, Tajima’s D , Fu’s F_S using Nei’s genetic distance, and the population parameter Θ_S were calculated in the R package *pegas* v1.1 (Paradis 2010). Estimates of time parameters and population parameters Θ_0 and Θ_1 (Table 2) were fitted to observed mismatch distributions to determine coalescence times, following Li (1977) and Rogers and Harpending (1992). Coalescence analysis requires an estimate of generation time and mutation rate. Generation time was estimated following Depczynski and Bellwood (2006) using age of maturity at 1 year and a maximum age of 10 years. Divergence estimates for cytochrome b at c. 2%/Myr between species and 1% within lineages have been obtained for a number of reef fishes (e.g., Muss et al. 2001), and this rate was provisionally applied here in the absence of a known rate for sardine. Demographic history and coalescence time were also analyzed by producing a Bayesian Skyline Plot (BSP) created in *Beast* v.2.7.6 (Bouckaert et al. 2019) using a GTR substitution model with 4 gamma categories and an in-run estimated shape parameter. 500×10^8 generations were run discarding the first 50×10^8 as burn in. In order to visualize the sequence data, a minimum-spanning haplotype network was created using *PopART* v.1.7 (Leigh and Bryant 2015; Bandelt and Röhl 1999). *Tracer* v.1.7 (Rambaut et al. 2018) was used to create the Bayesian Skyline Plot.

Results

On the one hand, overall haplotype diversity was large. Of the 434 individuals examined, 305 unique haplotypes were found ($h = 0.992$; GenBank accession #OR480110-OR480414). On the other hand, nucleotide diversity was low ($\Theta\pi = 0.0070$; Table 3). The most common haplotype was found in 29 individuals and was distributed across the entire sampling range, with the exception of Bahía Magdalena (Fig. 2). The largest haplotype diversity was observed at Guaymas, whereas the smallest haplotype diversity was seen at Vancouver Island (Table 3). Both

Table 3. Molecular diversities for 434 cytochrome *b* haplotypes. All tests of Tajima’s *D* and Fu’s *F_s* were significant < 0.05, the * indicates significance at <0.01. Site specific statistics and overall Tajima’s *D* and Fu’s *F_s* were calculated in Arlequin v3.5 (Excoffier and Lischer 2010), overall haplotype and nucleotide diversities were calculated in R with the “Nei” *D_a* in pegas v1.1, using the sum of the number of differences between pairs of sequences divided by the number of comparisons (Paradis 2010), an * indicates significance at <0.01.

Site	<i>n</i>	No. Haps.	Haplotype Diversity (<i>h</i>)	Nucleotide Diversity (Θπ)	Tajima’s <i>D</i>	Fu’s <i>F_s</i>
Vancouver Island	30	25	0.9816 ± 0.0162	0.007332 ± 0.003908	−1.98074	−13.92893*
Washington	33	29	0.9867 ± 0.0135	0.007647 ± 0.004049	−1.82993	−19.5419*
Oregon	49	46	0.9966 ± 0.0053	0.007069 ± 0.003727	−2.13141	−24.96678*
Crescent City	32	29	0.9919 ± 0.0110	0.006840 ± 0.003658	−1.90545	−22.52028*
Pescadero	38	34	0.9943 ± 0.0075	0.006177 ± 0.003317	−2.04119	−22.15685*
Central California	30	27	0.9862 ± 0.0158	0.007003 ± 0.003746	−2.06103*	−19.35412*
San Diego	30	28	0.9954 ± 0.0100	0.007284 ± 0.003884	−1.87118	−21.6095*
Ensenada	37	32	0.9910 ± 0.0090	0.005822 ± 0.003146	−2.15061*	−25.15392*
Isla Cedros	30	29	0.9977 ± 0.0094	0.007600 ± 0.004039	−2.17351*	−23.85028*
Bahía Magdalena	38	34	0.9929 ± 0.0084	0.007162 ± 0.003796	−2.17983*	−24.92506*
El Desemboque	40	35	0.9897 ± 0.0097	0.007170 ± 0.003794	−2.03867*	−24.9286*
Guaymas	47	46	0.9991 ± 0.0046	0.007003 ± 0.003699	−2.2677*	−24.98442*
Overall	434	305	0.99235	0.00703	−2.42001*	−24.22658*

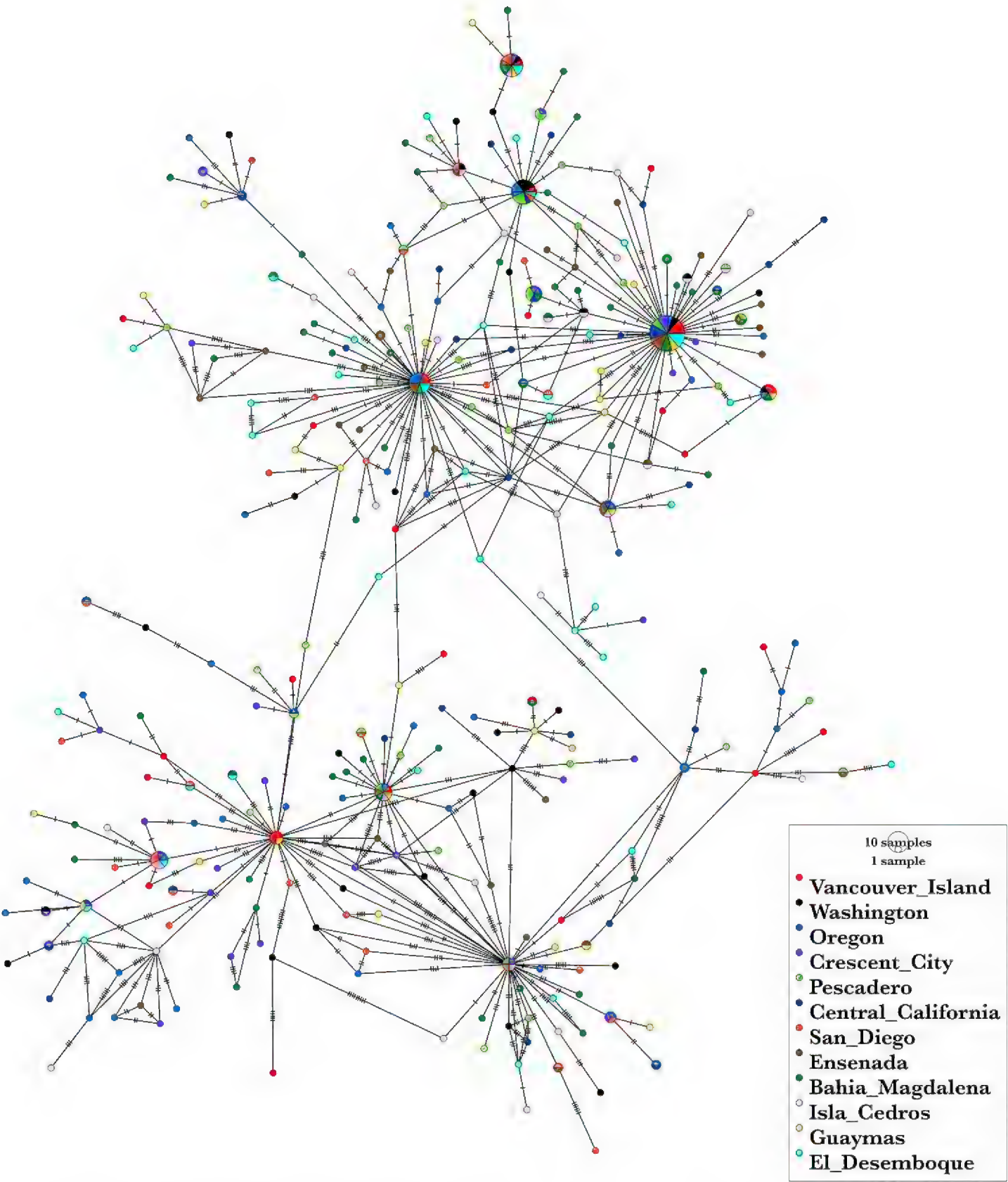


Fig. 2. Haplotype network for 434 individuals inferred by Minimum Spanning Network (Bandelt and Röhl 1999). Hash marks indicate single mutations between haplotypes.

Tajima’s D and Fu’s F_S were negative and significant when measured site by site, and was also negative and significant overall (Table 3). Θ_S was estimated at 37.74943.

Estimated coalescence time using the traditional mismatch method was approximately 235,400 years before present. The Bayesian Skyline Plot, however, identified two periods of rapid population growth, one beginning ~280 kya and a second beginning ~110 kya (Fig. 3). Present day effective population size (N_e) was estimated at nearly 100 million.

Φ_{ST} , the measurement of variance within sampling sites, showed small but significant values, and Φ_{CT} , which measures variance among groups, was small and non-significant when Bahía Magdalena was included in the Pacific group, but was significant when included in the

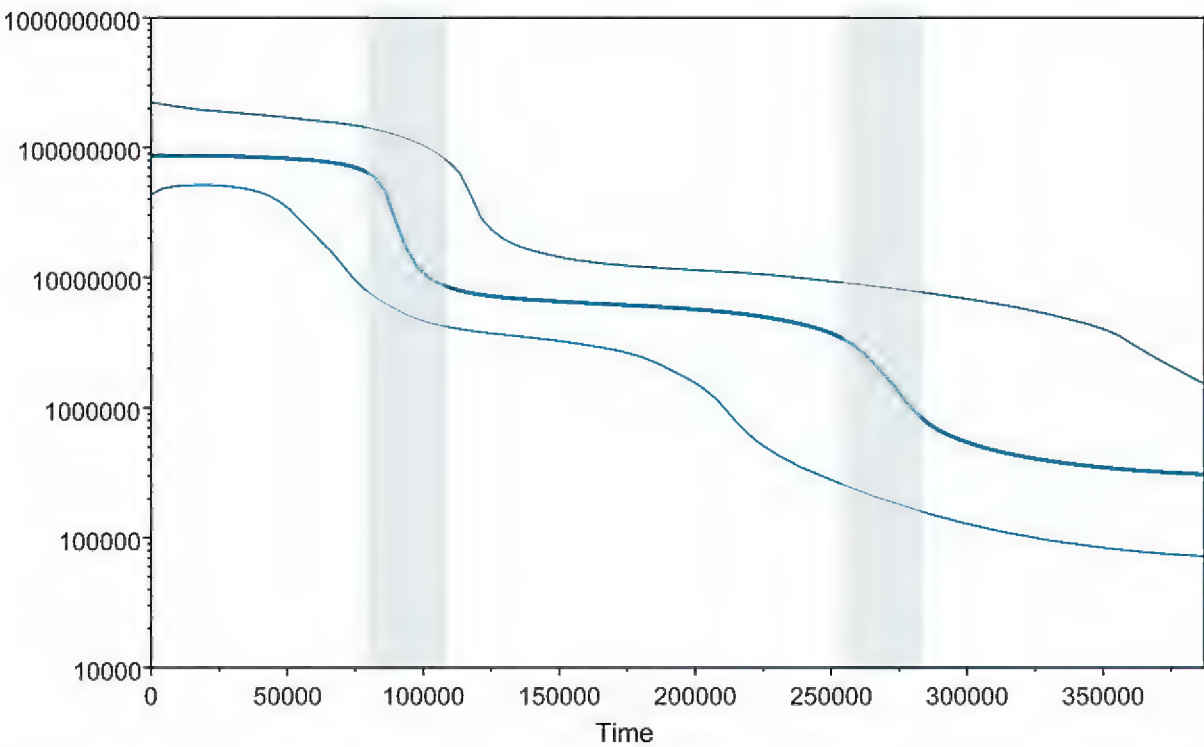


Fig. 3. Bayesian Skyline Plot created in Tracer v1.2 (Rambaut et al. 2018). The bold line shows the median population size, the two blue lines represent the upper and lower bounds of 95% highest posterior density interval. Expansion events are indicated between the vertical shaded lines. The x-axis represents time in years and the y-axis is on the log scale.

GOC group (Table 4). Pairwise Φ_{ST} values ranged from 0.0000 to 0.04487, with several significant but small values (Table 5).

Discussion

Throughout the studied range in the northeastern Pacific Ocean, biogeographic boundaries align with phylogeographic breaks in some demersal species, however these patterns differ among species (Bernardi 2000; Bernardi and Talley 2000; Dawson et al. 2001; Craig et al. 2011; Chabot et al. 2015). Many demersal species experience limits to gene flow related to habitat associations, although few appear constrained by Point Conception as phylogeographic breaks seem to align with other geographic boundaries, especially for fishes that are restricted to specific habitats or are live-bearing (Bernardi 2000; Bernardi and Talley 2000; Terry et al. 2000; Dawson et al. 2001; Craig et al. 2011). For example, the California halibut (*Paralichthys californicus*) does not show phylogeographic breaks across its studied range despite its relatively sedentary adult behavior. Strong connectivity likely results from the approximately 30-day pelagic egg and larval stage conferring sufficient dispersal ability to

Table 4. Fixation indices for the total of all samples, and for separate groupings of samples from the Pacific and the Gulf of California. The * indicates a grouping of all Pacific samples with Bahía Magdalena included in the Gulf of California group. Indices calculated using “raw” in the strataG v2.5.01 package for R (Archer et al. 2016).

Group	Φ_{ST}	Φ_{CT}
Total	0.01136, p = 0.032	-
Pacific/GOC	-	0.00435, p = 0.128
Pacific/GOC*	-	0.00923, p = 0.21

Table 5. Pairwise comparisons of gene flow between sampled locations represented by Φ_{ST} computed in Arlequin v3.5 (Excoffier and Lischer 2010). VI = Vancouver Island, WA = Washington State, OR = Oregon, CR = Crescent City, PES = Pescadero, CC = Central California, SD = San Diego, ENS = Ensenada, IC = Isla Cedros, BM = Bahía Magdalena, ED = El Desemboque, GY = Guaymas. An (*) indicates a significance level of less than or equal to 0.05. Negative values are reported here as 0.00000, given that negative values of genetic distance are artifacts of the software and are not biologically meaningful.

	VI	WA	OR	CR	PES	CC	SD	IC	ENS	BM	ED	GY
VI	0.00000	-	-	-	-	-	-	-	-	-	-	-
WA	0.00000	0.00000	-	-	-	-	-	-	-	-	-	-
OR	0.01023	0.01663	0.00000	-	-	-	-	-	-	-	-	-
CR	0.00392	0.00782	0.00000	0.00000	-	-	-	-	-	-	-	-
PES	0.00000	0.00000	0.02266*	0.02023	0.00000	-	-	-	-	-	-	-
CC	0.00000	0.00000	0.02743*	0.02282	0.00000	0.00000	-	-	-	-	-	-
SD	0.00000	0.00000	0.01578	0.00821	0.00000	0.00000	0.00000	-	-	-	-	-
IC	0.00838	0.01692	0.04415*	0.04800*	0.00000	0.00000	0.00000	0.00000	-	-	-	-
ENS	0.01219	0.00724	0.00000	0.00000	0.02431*	0.00000	0.00835	0.00845*	0.00000	-	-	-
BM	0.00000	0.00000	0.03658*	0.02862*	0.00000	0.00000	0.00000	0.00000	0.03197*	0.00000	-	-
ED	0.00256	0.00686	0.03450*	0.02666*	0.00000	0.00000	0.00000	0.00000	0.02561*	0.00000	0.00000	-
GY	0.00000	0.00000	0.01666*	0.00841	0.00000	0.00000	0.00000	0.00000	0.00925	0.00000	0.00000	0.00000

cross barriers (Craig et al. 2011). Given the mobility of the Pacific sardine as a pelagic fish and its broad habitat requirements, Point Conception is an unlikely barrier to gene flow for sardine which is borne out by the data herein.

The Punta Eugenia biogeographic boundary is a biogeographic break that coincides with a phylogeographic break for several fishes (Bernardi et al. 2003) and is hypothesized to have been connected to the current day Gulf of California by an ancient Neogene seaway (Riddle et al. 2000). The closure of this seaway is thought to have resulted in the present-day disjunct distributions of several marine fishes (Bernardi et al. 2003). These disjunct distributions may also have resulted from more recent dispersal of organisms around Cabo San Lucas during times of glacial cooling that is not possible under current climate conditions (Riddle et al. 2000; Bernardi et al. 2003). This is due to the presence of warm waters at the southern extreme of the Baja California Peninsula, which act as a barrier to temperate species (Bernardi 2000; Bernardi and Talley 2000; Bernardi et al. 2003). The possibility for ongoing connection by migration in deeper water or in cooler months, as well as the mobility and tolerance of a broad temperature range makes it unlikely that this biogeographic boundary limits gene flow for Pacific sardine which is supported by the data herein.

The small divergences between Pacific sardine populations support a lack of barriers to long-time scale gene flow in this motile pelagic species when compared to demersal fishes in the same geographic range. Both overall (Table 4) and pairwise genetic distances (Table 5) suggest substantial gene flow across the Point Conception and Punta Eugenia biogeographic boundaries as well as evolutionary time scale connectivity between the Gulf of California and the Pacific Ocean. The genetic analysis of the Pacific sardine shows no evidence of population structure and high genetic diversity across the sampled range which is consistent with previous studies (Hedgecock et al. 1989; Lecomte et al. 2004; García-Rodríguez et al. 2011). This is supported by large overall haplotype diversity ($h = 0.992$) and the large number of unique haplotypes. Among the 434 individuals, there were 305 unique haplotypes and a majority of shared haplotypes were only observed twice (Fig. 2). Nucleotide diversity was also low ($\Theta\pi = 0.0070$). Such patterns are consistent with previous findings for Pacific sardine, and indicate an evolutionarily recent population expansion of this population (Bowen and Grant 1997; Grant and Bowen 1998; Avise 2000). The coalescence time estimate of $\sim 235,400$ yr before present indicates an evolutionarily recent coalescence and agrees with previous estimates (Bowen and Grant 1997; Grant and Bowen 1998; García-Rodríguez et al. 2011). Other studies on pelagic populations of fishes which have shown large panmictic populations have similarly high haplotype diversities (Lecomte et al. 2004; Theisen et al. 2008; Zheng et al. 2015; Min et al. 2019).

Neutrality tests also support the notion that the population may have undergone a large and evolutionarily recent expansion. As with high haplotype diversity, negative Fu's F_S and Tajima's D have been consistently found in recent studies of panmictic pelagic fishes (Lecomte et al. 2004; Zheng et al. 2015; Min et al. 2019). A negative D indicates an excess of low-frequency polymorphisms relative to neutral expectation, which may reflect rapid population growth and can mean that the population has undergone a bottleneck in its evolutionary past. Nevertheless, it is important to remember that a selectively neutral site could be linked to a site under selection and this could cause negative values to appear in the absence of past bottlenecks (Tajima 1989). As cytochrome b is mitochondrial, it is inherited as an entire unit with the rest of the mitochondrion and therefore could be under any form of selective pressure that other parts of the mitochondrial genome would be experiencing. Fu's F_S was consistently negative and significant. Older mutations that occurred closer to the time of the most recent common ancestor are expected to occur more frequently, while younger mutations indicative of a recent expansion would be infrequent.

Negative values of Fu's F_S imply an excess of rare alleles at low frequency and may suggest a recent expansion (Fu 1997). The high value of Θ_S , which is positively correlated with population size, suggests a large effective population size. The estimated coalescent time agrees with previous work (García-Rodríguez et al. 2011) and concurs with a recent population expansion as indicated by the neutrality tests.

Another hypothesis suggests that high h and low $\Theta\pi$ may reflect “selective sweepstakes reproduction,” in which natural selection and the low probability of survival to adulthood (i.e., reproductive skew) combine to create a genetic pattern that also includes an excess of small mutations (Niwa et al. 2016; Árnason et al. 2023; Eldon and Stephan 2023). Given the biological nature of sardines, sweepstakes recruitment is expected, thus it is possible that both processes (rapid population expansion over evolutionary time scales and selective sweepstakes reproduction) have worked together to produce the current genetic architecture of Pacific sardine.

The BSP of population size shows two population expansion events, one beginning ~ 280 kya and a second beginning ~ 110 kya. We speculate that the ~ 280 kya event corresponds to the same event estimated at ~ 235 kya by the traditional coalescence approach. Population expansions in marine fishes are often considered in the context of changes in sea surface temperatures (SST) as they are reasonable indicators of suitable habitat. Around 400,000 yr BP, an increase in the amplitude of climate cycles across the globe known as the Mid-Brunhes Transition occurred. Interglaciation periods were warmer than before and resulted in warmer SST (Morley and Dworetzky 1991; Barth et al. 2018; Kender et al. 2019). Similar trends were present in the California Current, as interglacial warming periods and associated warmer sea surface temperatures have been observed by sedimentary core sample analysis (Heusser 1998; Lyle et al. 2010). Both of the expansion events identified by the BSP correspond to rapid increases in SST in the California current system following periods of SST below $\sim 13^\circ\text{C}$ (Herbert et al. 2001). Given that $13\text{--}16^\circ\text{C}$ is the optimal range for Pacific sardine egg and larval survival (Lasker 1964; Agostini et al. 2007; Dorval et al. 2019), we hypothesize that reproductive success during these cooler periods was reduced and prevented population growth.

Conclusion

Overall, the low but significant fixation indices support the null hypothesis of no genetic structure within the Pacific sardine of the northeastern Pacific that reflects evolutionary time scale processes. However, the significant values observed in the fixation between groups are worth reviewing, as significant differences between sampling groups were observed when Bahía Magdalena was grouped with the GOC (Table 4). This could imply that there is more similarity in the genetic architecture of Pacific sardine in Bahia Magdalena and the GOC. That said, spurious significance of small values of Φ_{ST} are common and may not be biologically meaningful (Waples 1998; Hedrick 1999; Meirmans and Hedrick 2011). Regardless of whether or not subtle structure exists between a Bahia Magdalena/GOC group and the rest of the Pacific, there is no evidence of phylogenetic structure north of Bahia Magdalena.

The molecular data presented here and the previous molecular studies on this species support the hypothesis of a single panmictic population. The data herein agree with the previous research and align with the traditional notion of substantial gene flow between pelagic fish populations. In the absence of data to the contrary, it is reasonable to accept the hypothesis that there is no genetic structure in the Pacific sardine in the northeastern Pacific Ocean. Mitochondrial studies will not reflect a recent separation as there is not enough time for lineage

sorting (Avice 2000), and future genetic studies using different markers or analyses may yield different results and would expand our understanding of the genetic architecture of Pacific sardine in their northeast Pacific range.

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Ecology and Conservation Status of Morro Manzanita *Arctostaphylos morroensis*: A Threatened Plant Endemic to Los Osos, San Luis Obispo County, California

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Abstract.—Morro manzanita *Arctostaphylos morroensis* (Ericaceae) is a long-lived, shrub endemic to San Luis Obispo County, southern California, USA. It was listed as threatened under the U.S. Endangered Species Act in 1994, with identified threats being residential and urban development, including lack of protection on private land and lack of management on public lands, competition with invasive non-native plants, and risks of extinction associated with small and isolated populations. Our goal in this paper is to summarize and supplement the current knowledge of Morro manzanita. We review the literature on the species' description, reproductive ecology, germination cues, short-term response to fire, and distribution. We conducted field surveys to report on long-term response to fire, resampling the previously studied prescribed burn site 25 yr post-fire. Finally, we summarize the current land management of sites that support Morro manzanita and threats faced by this species. We conclude with specific recommendations for management and future study towards supporting conservation of this species and its maritime chaparral community.

Manzanitas, genus *Arctostaphylos* (Ericaceae), are iconic shrubs in the California chaparral, even where they are not numerically dominant. Nearly all taxa in this genus are found within the California Floristic Province, and 60% are local endemics (Kauffmann et al. 2021). Most of these are components of maritime chaparral, a plant community that is itself of special interest and recognized by the California Coastal Commission as an “Environmentally Sensitive Habitat Area” (Kauffmann et al. 2021). Some restricted endemic manzanita species have experienced significant range reductions and persist in stands that are fragmented and facing various threats related to human development; seven of these are listed as threatened or endangered under the U.S. Endangered Species Act.

Morro manzanita *Arctostaphylos morroensis* is a long-lived, perennial shrub endemic to San Luis Obispo County, southern California, USA. The species was listed as threatened under the U.S. Endangered Species Act in 1994 (USFWS 1994). It is recognized also as a 1 B.1 rare plant (seriously threatened) by the California Native Plant Society.¹ Morro manzanita is an obligate seeder, meaning that it does not resprout from a burl following fire, but rather maintains its populations solely by regenerating from soil-stored seedbanks. Previous studies suggest that fire plays a crucial role in establishment and persistence of this species (Odion and Tyler 2002; Tyler and Odion 2020).

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¹ California Native Plant Society, Rare Plant Program. 2023. Rare Plant Inventory (online edition, v9.5). <https://www.rareplants.cnps.org> [accessed 9 January 2023].

The historical range of Morro manzanita was estimated to comprise 800 to 1,100 ha (USFWS 1994). However, at the time of listing in 1994 the area of occupancy was estimated to remain at only 340 to 360 ha (Mullany 1990)², with estimates of number of individuals ranging from 86,000 to 153,000³. Some of the data cited to describe the range of the species, particularly at the edges of the distribution, have not been verified for decades, suggesting that new surveys are warranted and could be of value. In addition, an understanding of current land use and status could help understand potential for long-term persistence of the species. For example, at the time of listing, ~65% of the habitat was on private lands with no legal protection. The remaining 35% was on lands owned by California State Parks [CSP] and California Department of Fish and Wildlife [CDFW], and it was comprised of stands with low densities, possibly representing only 20% or less of total individuals. Information on present range and land use could provide an updated overview of threats to this species. In 1994, much of the habitat was slated for development or alteration (USFWS 1994). The identified threats were residential and urban development, including destruction and fragmentation of habitat, lack of protection on private lands and lack of management on public lands, and deterioration of habitat due to recreational activity. Other concerns were expansion of previously cultivated non-native *Eucalyptus* into habitat (Mullany 1990) and invasive plants, as well as stochastic extinction by virtue of the small and isolated nature of the remaining populations (USFWS 1994).

Research has been conducted on the reproductive ecology (Tyler et al. 2023), germination cues and seedbank dynamics (Tyler and Odion 2020), and early responses to prescribed burning (Odion and Tyler 2002). A comprehensive summary and evaluation of these findings could help guide present management of this species. In particular, recommendations on the role of burning are needed and require information about the longer-term recovery from prescribed fire. The studies reported in Odion and Tyler (2002) followed seedbank dynamics and seedling recruitment after fire in a 40-yr-old stand of Morro manzanita. They found that three years after the prescribed burn, seedling density was still very low and the abundance of individuals in the stand was less than half that of the pre-burn population. Their findings suggested that the stand may have experienced an overall decline after this burn. However, for a long-lived species such as Morro manzanita, postfire recovery and community succession may not be evident for decades. For this study we present recent data collected in the original burn area to report on recovery 25 yr after the prescribed fire.

The goal of this paper is to summarize and supplement the current understanding of the biology and conservation status of Morro manzanita, including its distribution, abundance, ecology, threats, and management. We review the existing literature and report new findings from recent field surveys. We conclude with recommendations for management and future study towards supporting conservation of this species and its maritime chaparral community.

Materials and Methods

For our literature review and associated discussion, we reviewed all available sources including published scientific journal articles, federal and state agency reports, descriptions in published taxonomic keys, academic theses and dissertation, and information in the California

² LSA Associates, Inc. 1992. An assessment of the status of the Morro manzanita (*Arctostaphylos morroensis*). Prepared for Central Coast Engineering, San Luis Obispo, California. Irvine, Calif., 9 pp.

³ Crawford, Multari, Clark Associates. 2004. Los Osos Habitat Conservation Plan, Species Accounts, Appendix D [draft]. Prepared for Los Osos Community Services District, San Luis Obispo County, California. San Luis Obispo, Calif., 309 pp.

Natural Diversity Database and California Native Plant Society's rare plant inventory. In describing the distribution of Morro manzanita, we considered a location with the species as a separate occurrence if >0.4 km from the nearest occurrence (CDFW 2024). Latitude, longitude and elevation were determined using Google Earth aerial imagery and tools, or a global positioning system (GPS) device in the field. Stated areas of properties are from records of the County of San Luis Obispo. We discuss each occurrence in sequence according to its assigned number in the California Natural Diversity Database (CDFW 2021). Common and scientific names of plants follow those used in Jepson eflora⁴. Field surveys were conducted in January, February and November 2023. We visited the reported occurrences in attempt to confirm the presence of Morro manzanita. We also surveyed additional locations that we hypothesized could have the species based on edaphic conditions (e.g., 2.2 km east of occurrence 20 in an area with Baywood fine sand).

In order to improve our understanding of postfire recovery, we resampled the stand in which a prescribed burn had been conducted in November 1998 in Montaña de Oro State Park. In that area, transects and vegetation plots had been established and sampled prior to the burn (September 1998), and after (November 1998, April 2000, April 2000, June 2001). In addition to seedbank collections and assessment of fire severity, percent cover of Morro manzanita and other plant species was recorded within 2 m radius circular plots. In March 2023, we relocated the original vegetation transects and resampled twelve of the original thirteen plots that had contained $>94\%$ cover of Morro manzanita prior to the burn. We compare these findings with those reported from the period 1 to 3 yr postfire.

Results and Discussion

Description.—Wieslander and Schreiber (1939) first named and described Morro manzanita, referencing specimens collected in 1936 and 1938 in and near Hazard Canyon south of Morro Bay, an area now within Montaña de Oro State Park. The species name was based on the locality where it occurs. The three collections, including holotype, are filed in the Vegetation Type Map Herbarium at the University of California, Berkeley (Wieslander and Schreiber 1939). Additional specimens used by Wieslander and Schreiber (1939) to verify the range are in the herbaria at Stanford University and the California Academy of Sciences. Other taxonomic descriptions, briefly summarized here, include Hoover (1970), Wells (2000), Parker et al. (2012), and Kauffmann et al. (2021).

Morro manzanita is an erect spreading shrub, generally 1 to 4 m in height (Parker et al. 2012), with some older individuals reaching 8.5 m tall (Fig. 1). This manzanita species lacks a basal burl, which is both a distinguishing taxonomic characteristic, and indicative of its postfire recovery response (Jepson 1916; Wieslander and Schreiber 1939; Keeley and Zedler 1978). Other distinctive morphological traits include its bark and leaf morphologies. The grayish-brown bark on mature stems is shreddy but persistent. Its leaves are oblong to oblong-elliptic (1.5 to 3 cm long), truncate to subcordate at the base (not auriculate-clasping), with short petioles (2 to 5 mm) (Fig. 2). Notably, the leaf surfaces are unlike: dark green, shiny and lacking stomata above, while gray-tomentose on the lower surface (Hoover 1970; Wells 2000; Parker et al. 2012). Wells (1968) determined that Morro manzanita has a base chromosome number of 13 and it is diploid ($2n = 26$).

⁴ Jepson Flora Project (eds.) 2024. Jepson eFlora, <https://ucjeps.berkeley.edu/eflora/>



Fig. 1. Morro manzanita: top, in coastal maritime community at Montaña de Oro State Park; and bottom, tree-like individual on the north-facing slopes of the Morro Dunes Ecological Reserve Bayview Unit. The person standing in the bottom photo is 1.86 m tall. Photos by the authors.



Fig. 2. Morro manzanita *Arctostaphylos morroensis*: upper left, flowers; upper right, living fruits; bottom, fruits and seeds collected mostly from the litter seedbank. Upper photos by Michael Walgren, Calif. State Parks, San Simeon. Bottom photo by the authors.



Fig. 3. Distribution of Baywood fine sand (shown in yellow) in the Los Osos area, San Luis Obispo County, California. Map credit: Mark Metevier, USFWS.

Abiotic conditions.—Morro manzanita occurs near the coast at elevations <200 m (Parker et al. 2012), primarily on stabilized sand dunes of Baywood fine sand (Fig. 3) (Carpenter and Storie 1928; Soil Conservation Service 1984; Wiegers 2009), with few outlying locations on outcrop shale or volcanic igneous substrate. The climate is Mediterranean, with cool moist winters and warm dry summers. Fog is common. Temperatures range from ~6.5° to 23.5°C, and mean annual rainfall (recorded at Morro Bay Fire Station) is 42.1 cm, with 75% occurring between November and April.

Fog has been found to play a key role in determining the composition of maritime chaparral community structure (Vasey et al. 2014) and the physiological performance of *Arctostaphylos*

species in particular (Vasey et al. 2012). Long-term data sets in combination with modeling suggest that there has been a significant reduction in fog since the early 1900's (Johnstone and Dawson 2010), which could have particularly negative consequences for obligate-seeding manzanitas in maritime chaparral (Vasey et al. 2012).

Associated plant communities.—Weislander and Schreiber (1939) noted that Morro manzanita was associated with chamise *Adenostoma fasciculatum*, black sage *Salvia mellifera*, California goldenbush *Ericameria ericoides*, and deerweed *Lotus scoparius* (now *Acmispon glaber*). To quantify descriptions of associated plant communities, Tyler and Odion (1996) surveyed mapped polygons, or areas, where Morro manzanita was shown to occur (Mullany 1990). In a subset of these (24 locations total) and over a range of manzanita cover classes, they recorded the plant species present within 100 m² circular quadrats using the relevé technique and then used two-way indicator species analysis to detect species associations. They found the vegetation in areas where living Morro manzanita occurs consists of four recognizable types: maritime chaparral, coastal scrub, pure stands of Morro manzanita, and stands of Morro manzanita dominated by coast live oak *Quercus agrifolia* (Tyler and Odion 1996). Much of the natural landscape in Los Osos is dominated by maritime chaparral, a relatively rich assemblage of chaparral shrubs, subshrubs, and herbaceous plants, with considerable bare ground. In maritime chaparral associations, in addition to manzanita, the dominant and indicator species of this vegetation are California goldenbush, chamise, wedgeleaf ceanothus *Ceanothus cuneatus*, bush monkey flower *Mimulus* (now *Diplacus*) *aurantiacus*, and deerweed (Tyler and Odion 1996). Although these plants occur among and immediately adjacent to Morro manzanita, they usually do not occur underneath its canopy. Areas adjacent to the manzanita also have substantial areas of bare ground, generally 30 to 50%. With openings and bare sand between shrubs, this maritime chaparral is characterized by a considerable herbaceous flora that varies significantly from year to year depending on rainfall. Where this community has been mechanically disturbed, chaparral species abundance is much lower, and coastal scrub species, such as California sagebrush *Artemisia californica*, black sage, coyote bush *Baccharis pilularis*, dune bush lupine *Lupinus chamissonis*, and yellow bush lupine *L. arboreus*, dominate, including a host of annual and perennial native herbs, and non-native species.

Embedded within these associations are patches of pure Morro manzanita stands. On level sites, such as the Elfin Forest Preserve in north Los Osos, the patches of live manzanita are relatively small, consisting of one to a few usually large individuals, with diameters at the base (db) up to 1 m, heights over 7.5 m, and canopy diameters up to ~10 m. The sloping landscapes to the south and southwest of Los Osos, around Hazard Canyon, support large patches of nearly continuous Morro manzanita covering many hectares. Both large and small patches of pure manzanita are characterized by having few associated species, such as California manroot *Marah fabaceus* and bush monkey flower (Tyler and Odion 1996). Also embedded within the maritime chaparral and coastal scrub associations were *Quercus* dominated patches, occasionally near or adjacent to pure Morro manzanita patches. These were characterized by high relative abundances of three understory species: California manroot, fuchsia-flowered gooseberry *Ribes speciosum*, and poison oak *Toxicodendron diversilobum*. This vegetation type differs from the dense *Quercus* forests found on steep north facing slopes in the area (e.g., on the north-facing slope just north of the Elfin Forest). The factors influencing species composition in the community types hosting Morro manzanita have not been investigated, but likely include soil characteristics, fog frequency, patch size, time since fire, and extent of soil disturbance or mechanical clearing.

Lifespan.—Morro manzanita is long-lived, and while maximum lifespan has not been reported in the literature, Tyler and Odion (1996) estimated stand ages using historical aerial photographs from the collection in the Map and Imagery Library at the University of California, Santa Barbara. They examined images from 1949 to 1992 to identify areas that had been cleared and/or burned. In addition, cross-sections of co-occurring wedgeleaf ceanothus were collected from the areas where stand age was estimated; this species is an obligate seeder and thus would have germinated following fire, at the same time as the manzanitas present in the stand. The annual ring counts from cross-sections confirmed the minimum stand ages based on aerial photos. In 1996, the stand ages ranged from 37 to >47 yr old, with the large tree-like (arborescent) individuals in the Elfin Forest Preserve estimated to be significantly older than 47 yr; as described above, some of these individuals are exceptionally large. Since that 1996 report, there has been only one fire in all the sites surveyed, a prescribed burn conducted in 1998 within a stand containing Morro manzanita. Thus, at present the youngest stand is 25 yr old, and the oldest stand is a minimum of 74 yr old (though most likely much older).

Flowering and fruiting.—One of the distinctive and distinguishing characteristics of species in the genus *Arctostaphylos* are the “nascent” or immature inflorescences, developed many months before flowering (Jepson 1938; Keeley 1997). In Morro manzanita, these immature panicles are pendent and campanulate (Weislander and Schreiber 1939; Parker et al. 2012). Small urn-shaped flowers, which are white and occasionally tinged with pink, appear in January through March (Fig. 2).

Similar to other obligate-seeding manzanita species (Keeley 1977; Fulton and Carpenter 1979; Mahall et al. 2010), Morro manzanita produces abundant flowers. Tyler et al. (2023) recorded an average of 50 to 135 flowers per stem across a two-year period. Flower production (number of flowers per stem) varies among sites and among years with much higher (two times the average) flower production across sites in a very wet year (1998) compared to a year with below-average rainfall (1999) (Tyler et al. 2023). Water availability has been found to be related to both flower and fruit production in other manzanitas, though the timing of rainfall that influencing production varies between species. For big berry manzanita *A. glauca* and Eastwood manzanita *A. glandulosa* (Keeley 1977), as well as *A. visida* (Baker et al. 1982) resources (water) in the previous year appear to determine flower production. However, Mahall et al. (2010) suggested that *A. glauca* flower production was influenced by rainfall in both the previous year, when buds are formed, and in the present year, when flower and fruit development occur. Observations of pointleaf manzanita *A. pungens* (Richardson and Bronstein 2012) and Morro manzanita (Tyler et al. 2023) indicate that flower production was most strongly related to present year resources (rainfall).

Reproduction of Morro manzanita is dependent on pollinators. Tyler et al. (2023) found that when inflorescences were bagged to exclude animal pollinators, fruits were not produced. Bees are the most common pollinators of Morro manzanita, and include yellow-faced bumblebees *Bombus vosnesenskii*, the common anthophorid bee *Anthophora urbana*, halictid bees, *Colletes* sp., and European honey bees *Apis mellifera*. Other pollinators observed visiting Morro manzanita flowers include syrphid flies, monarch butterflies *Danus plexxipus*, bee flies *Bombylius* sp. and Anna’s hummingbird *Calypte anna* (Tyler et al. 2023). This is consistent with research on congeners that demonstrated self-incompatibility and reliance on pollinators for successful reproduction – pink bracted manzanita *A. pringlei* var. *drupacea* and *A. glauca* (Brum 1975) and *A. pungens* (Richardson and Bronstein 2012). Bees have also been found to be important pollinators of other manzanita species (Gankin and Major 1964; Brum 1975; Fulton and Carpenter 1979).

Fruit set, or the percent of flowers producing a fruit, varies among sites and years, ranging from an annual average of 10 to 18% (Tyler et al. 2023). These data were reported for two adjacent years (1998, 1999), with fruit set being consistent across years for some sites, and varying significantly (5 times higher in one year) at another site. To our knowledge, the only other reported data on fruit set in manzanitas are for *A. pungens* (Richardson and Bronstein 2012), in which highest values for fruit set in control/natural conditions was 36% in 1998, and no fruit set observed in the following year. Thus, although we suspect fruit set is comparatively low in Morro manzanita, longer-term data and data on other congeners are lacking in order for this to be confirmed. Seed set, as determined by viable seed to ovule ratios, has been investigated in several species of manzanita by Kelly and Parker (1991). They reported that Morro manzanita has an average of 7.3 ovules per ovary/fruit (each flower contains one ovary in the Ericaceae), and that seed set is relatively high at 73% (Kelly and Parker 1991). This suggests that once pollinated, Morro manzanita successfully produces viable seeds, and that low fruit set may indicate pollinator limitation (Tyler et al. 2023).

The fruits mature in spring-summer (Fig. 2). They are reddish-brown and spherical to slightly flattened, or depressed globose. Morro manzanita fruits are drupes, covered by a thin exocarp, and containing a dry, mealy mesocarp surrounding multiple hard stones or “nutlets” (Meyer 2008; Parker et al. 2012). They contain an average of five (Kelly and Parker 1991) to eight (Tyler and Odion 1996) generally unfused nutlets (i.e., seeds) per fruit. Fruit drop occurs in late spring to late fall, though the timing can vary annually. Tyler et al. (2023) found that the majority of fruits fell from the plants during June and early July one year (1998), and August to early October in the following year (1999).

Fruit predation.—Dropped fruits of Morro manzanita are removed quickly by predators. Tyler et al. (2023) conducted studies of removal rates by vertebrate predators, by placing trays containing mature fruits under and adjacent to shrubs in several different manzanita stands, and under multiple individual shrubs. This was carried out in two years (1998, 1999) and was initiated when natural fruit drop was first recorded. They found that in both years, predators, most likely small mammals and birds, removed a majority of fruits and did it relatively quickly. From 60 to 70% of fruits were removed within 1.5 mo in both years. High predation rates have been reported for other species of manzanita (Keeley 1977; Kelly and Parker 1990).

Fruit removal alone does not mean all seeds are eliminated from the site, as some animals may scatter-hoard or cache seeds that could be incorporated into the soil seed bank (Parker 2015; Crowe and Parker 2023). Although no field studies have been conducted to identify the species that collect, bury, or consume fruits and seeds of Morro manzanita, Tyler et al. (2023) report observations of woodrat *Neotoma fuscipes*, and brush rabbit *Sylvilagus bachmani* feces in or adjacent to fruit trays. Morro Bay kangaroo rat, *Dipodomys heermanni morroensis*, potentially a former scatter-hoarder of Morro manzanita, has not been observed in the wild since 1986, and may be extinct (Villablanca et al. 2021). Other rodents, such as deer mouse *Peromyscus maniculatus*, brush mouse *P. boylii*, California pocket mouse *Chaetodipus californicus*, as well as birds that co-occur at the sites may collect, bury, or consume Morro manzanita fruits. Trail cameras set near fruit trays would be a useful method to identify associated animal species that interact with Morro manzanita, and help to assess the potential role they play in consumption vs. caching of seeds. Previous studies have documented the important role of scatter-hoarders in developing manzanita seedbanks (Parker 2015; Crowe and Parker 2023). However, in Morro manzanita it is unknown if such a mutualistic relationship exists, and some evidence points to consumers as seed predators rather than planters. For example, sites where seed bank density is exceptionally low (Elfin Forest) have the highest rates of fruit

removal (Tyler and Odion 2020; Tyler et al. 2023). In addition, relatively few Morro manzanita seeds are found in the soil away from the shrub canopies, and overall viable seed densities can be very low (Tyler and Odion 2020), suggesting that removal of a large fraction of fruits, even if some were buried and forgotten, could have a negative impact overall.

Seed input and seed banks.—Based on seed drop, seed predation rates, and estimates of the number of seeds per fruit for Morro manzanita, Tyler et al. (2023), estimated annual seed input to the soil seed bank over two years (1998 and 1999). The relative addition of seeds across years was similar at all sites (i.e., about 1.5 times greater in 1998 compared to 1999). However, seed input varied considerably among sites, and rates appear to decline with stand age. Annual seed input was at least 4 times lower at the oldest-aged stand at the Elfin Forest (316 seeds per m² in 1998) compared to the youngest stand in Montaña de Oro State Park (1,608 seeds per m² in 1998). The intermediate-aged stand had an intermediate value with an estimated seed input of 912 seeds per m² in this same year.

Tyler and Odion (2020) examined soil seed banks in different-aged stands, predicting that seed densities would be positively correlated with stand age. Soil cores (10 cm depth) were collected under multiple shrubs across three sites. Morro manzanita seed density in the soil varied greatly among sites, from 1,326 to 62,251 seeds per m². However, contrary to expectations, the oldest had especially low seed densities (Tyler and Odion 2020). Since seed input, as described above, was particularly low in the oldest stand, seed densities in the soil seedbank may decline even further. There has been one other study (Parker and Ingalls 2022) that reported seed bank densities of Morro manzanita. In their comparative study of ten *Arctostaphylos* species to investigate the relationship between seed size and seed bank density, Parker and Ingalls (2022) collected Morro manzanita seed and estimated the seed bank density (for 5 cm deep cores) to be an average of 1,900 per m². Although the location of their collections is not reported, if this value is doubled to make an equivalent comparison to results reported by Tyler and Odion (2020), their finding of a seed density of ~3,800 per m² is within the same range.

Interestingly, the striking variation in Morro manzanita soil seedbanks across different aged sites is in contrast to a previous study of change in manzanita seedbanks over time. Over a ten-year period, Keeley (1987a) found that despite the huge input of seed, estimated to be over 22×10^6 seeds per ha for the obligate-seeder *A. glauca*, no significant change in seed density was detected in the soil seedbank. Such an equilibrium in seed densities may be a result of input balanced against loss due to mortality factors including seed predation (Keeley 1987a). In contrast, the variation noted in Tyler and Odion (2020) in stands of Morro manzanita suggests a non-equilibrium. This difference may be, in part, related to stand age. The *A. glauca* individuals sampled in Keeley (1987a) were in a stand that was mature at 90 to 100 yr old. The Morro manzanita stands sampled in Tyler and Odion (2020) were ~40, >47, and >75 yr old, with accumulation of seed still occurring in the youngest stand, and net loss occurring in the oldest (potentially senescent) stand. Further study is warranted to investigate change in soil seedbanks over time. In addition, understanding the dynamics of fruits and seed accumulation in the litter layer (Fig. 2) would be useful as this may be a potential seed source for restoration efforts.

Seed viability and germination cues.—Percent viability of Morro manzanita seeds, i.e., the proportion of viable to total seeds, within the soil seed bank is very low across all stands, with an average of 4% (Tyler and Odion 2020). The oldest stand (the Elfin Forest) has exceptionally low seed viability, averaging 2%. Viability of fresh seeds has not been recorded in the literature, so rates of change with seed age are unknown.

Morro manzanita is an obligate-seeder, meaning that it does not resprout when the crown and stem are burned, and thus must re-establish from seeds in the soil seed bank. Seeds of obligate-seeders are mainly or even completely refractory (Sweeney 1956; Keeley 1987b; Keeley 1991); that is, germination is inhibited until primary dormancy is released by a specific mechanism. Fire-related cues such as heat and by-products of combustion have been identified as principal mechanisms that break seed dormancy of fire recruiters that rely on soil-stored seed (Keeley 1991). In other manzanita species, germination rates are low, but enhanced with smoke, charate, heat shock, or some combination of these treatments (Odion 2000; Keeley et al. 2005; Jurado et al. 2011). Tyler and Odion (2020) examined germination of Morro manzanita seeds in response to various cues, and confirmed that germination, while very low on average (1 to 4%), was greatest in treatments that combined heat and charred wood. However, neither heat nor charred wood alone enhanced germination (Tyler and Odion 2020). One factor responsible for low germination is that seed viability is very low – from 3 to 6%. Germination as a percentage of estimated viable seeds was found to be relatively high (23 to 100%). Unexpectedly, ~40% of viable seeds germinated with no fire treatments. Viability and germinability of fresh Morro manzanita seed has not been investigated. Although fresh and litter-stored seeds are unlikely to contribute to post-fire seedling establishment, as they would be consumed in a burn, this seed source might be used in restoration purposes; thus, study of its viability rates and germination cues is warranted.

Short-term response to fire.—To assess the effects of burning on seedling establishment in Morro manzanita, a prescribed fire was conducted by California State Parks in Montaña de Oro State Park in 1998. At the time of the burn, the stand was 40 yr-old. The total area burned was ~2.3 ha. Odion and Tyler (2002) recorded pre-burn seed densities and seed viabilities, seed mortality due to fire, and postfire germination and seedling survivorship for three years, to compare the population that established after the burn with the one present before.

Prior to the burn, there was abundant soil-stored seed, ~11,000 seeds per m², though seed viability in this stand (and all others) was low, resulting in an average of 334 viable seeds per m² before the fire. As was expected, seed mortality through the burn was high, and following the experimental fire only about a third remained, leaving an average of 99 viable seeds per m². Germination was recorded in the first two wet seasons after the fire, though most seedlings did not survive their initial summer drought. After three years, the density of Morro manzanita seedling was less than half the estimated density of the adult shrubs present before the fire. The authors speculated that the most likely factors responsible for the low number of seedling recruits were low numbers of viable seed in the soil, and relatively high mortality of both germinants and young seedlings. They concluded that with further mortality of the remaining seedlings and no additional germination, the 40-yr-old stand may not have had an adequate seed bank to compensate for mortality and thus prevent population decline.

Long-term recovery from fire: new findings.—Post-fire succession in maritime chaparral unfolds over many years and even decades. Early stages include the first years dominated by herbaceous annual and perennial species, resprouting shrubs, and seedlings of both woody sub-shrub species and shrubs (Keeley et al. 1981; Davis et al. 1988, 1989; Tyler 1996; Odion 2000; Odion and Davis 2000; Van Dyke et al. 2001; Fox and Potts 2023). Thus, a comprehensive view of post-fire vegetation patterns in chaparral is ideally based on observations over multiple time intervals.

In order to improve our understanding of long-term post-fire recovery of Morro manzanita, we resampled the stand in which a prescribed burn had been conducted in November 1998 in Montaña de Oro State Park. In that area, transects had been established and sampled prior to

Table 1. Pre- and postburn percent cover of woody species in 12 plots established in dense Morro manzanita *Arctostaphylos morroensis* prior to fire. Data are means for 12 circular plots (12.6 m² each). Vegetation cover was recorded 2 mo before the burn, and again 3 and 25 yr after the fire. Results for 1998 and 2001 from Odion and Tyler (2002).

Species	Preburn (Sep 1998)	3 yrs postburn (Jun 2001)	25 yrs postburn (Mar 2023)	
	mean	mean	mean	se
<i>Arctostaphylos morroensis</i>	99.5	<1	83.3	5.7
<i>Artemisia californica</i>	<1	7	0.7	0.4
<i>Baccharis pilularis</i>	0	4	0.6	0.4
<i>Ceanothus cuneatus</i>	0	<1	2.1	1.3
<i>Ericameria ericoides</i>	0	<1	0.2	0.2
<i>Eriophyllum confertiflorum</i>	<1	1	0.0	0.0
<i>Acmispon scoparius</i>	0	5	0.2	0.1
<i>Diplacus aurantiacus</i>	<1	2	0.6	0.4
Bare ground	<1	63	7.1	4.0

the burn (September 1998), and after (November 1998, April 2000, April 2000, June 2001). In addition to seedbank collections and assessment of fire severity, percent cover of Morro manzanita and other plant species was recorded within 12.6 m² circular plots (radius 2 m). These plots were located from 0 to 10 m off the transect (distance along and from the transects was recorded) and centers marked with rebar stakes. In the three years post-fire, these plots were easily relocated. Twenty-five years later, in March 2023, we identified the locations of the original vegetation transects. Unfortunately, the precise location of the original 12.6 m² circular plots could not be verified, as the center stakes were not evident and the area along the transect was now densely vegetated. However, given the certainty of the transect locations and using the recorded distance along and from the transects for the plot center locations, we are confident that we were within a few meters of the original plot locations. We resampled twelve of the original plots that had contained >94% cover of Morro manzanita in 1998 prior to the burn. We compare these findings with those reported from the period 1 to 3 yr postfire.

In the plots that had been dominated by Morro manzanita prior to the burn, the standing vegetation was almost entirely consumed in the fire (Odion and Tyler 2002). Three years later, the percent cover of all species was still low (~20% total) and the site was predominately bare ground (Table 1) Morro Manzanita seedlings were present but in low density, and cover of this species was still <1% on average. The plant species with the highest cover at this time were California sagebrush (7%), deerweed (5%) and coyote bush (4%) (Table 1). Based on these observations, and low manzanita seed densities in post-fire soil seedbank samples, Odion and Tyler (2002) reported concerns that the stand of Morro manzanita might not return to its pre-burn abundance or cover, and that the fire had caused a net loss to the population.

However, 25 yr post-burn we found that cover of Morro manzanita along the original transects was high, ranging from 30% to 100%, with a median of 93%, and mean of 83% cover Morro manzanita (Table 1). There was also a relatively low cover of associated species, including the obligate-seeding wedgeleaf ceanothus, which had been present at the site but was not found in these plots before the fire (Table 1). We observed that most manzanitas were flowering and/or producing fruit, indicating that individuals had reached sexual maturity and that replenishment of the soil seedbank is very likely taking place. These new findings suggest that this stand is recovering slowly but successfully from the fire, in terms of percent cover of

Morro manzanita. Conclusions based on data collected in the early post-burn period had suggested otherwise because of the low density of seedlings established by the third year after fire (Odion and Tyler 2002).

For several reasons, we propose that this significant increase in cover of manzanita (from <1% to 83%) is most likely the result of growth and increase in the canopy spread of seedlings that established within the first two years following the burn. Many obligate-seeding species such as Morro manzanita have refractory seed, which is stimulated to germinate by fire. In laboratory studies, Tyler and Odion (2020) found that highest seed germination in Morro manzanita resulted from the treatment that mimicked conditions of burning – heat and application of charred wood. In addition, young individuals of Morro manzanita are uncommon in unburned areas, supporting the hypothesis that it is the direct effects of fire that stimulates a flush of germination soon after the burn. Finally, Odion and Tyler (2002) compared viable seed densities in the soil before and one week after the burn, and found that in the top 5 cm of the soil less than a quarter of the viable seed remained (54 viable seeds per m²). They hypothesized that most of the seed that survived the fire would have been stimulated to germinate, which would have further depleted the seedbank.

Alternatively, additional seed germination in the third winter following the fire could have supplemented the number of seedlings establishing in this site. If this occurred, shrub densities may have approached those in the adult stand prior to the burn, and with time and increased growth, the canopy cover would also be restored. As noted above, most of the remaining viable seed would have probably germinated within the first two years of the burn. However, a small portion may have still been present after the germination that occurred in year two. Although previous studies on manzanita seed suggest that once dormancy is broken (by heat, scarification, or combustion products) germination is imminent (Keeley 1987b; Parker and Kelly 1989; Keeley 1991), Odion and Tyler (2002) recorded seedlings that emerged in the second year following the burn. The latter indicates that there is some mechanism by which treated (i.e., burned) seed may maintain dormancy for at least one year. It is unknown if this latent condition might extend even one more year post-burn.

Our 2023 field surveys provide evidence that, contrary to the conclusions of Odion and Tyler (2002) that the former population of Morro manzanita had failed to re-establish after the fire, reestablishment of manzanita cover is occurring in this site. While this stand was relatively young (40 yr old) at the time of the prescribed fire, seedbank densities and subsequent seedling recruits may have indeed been adequate to restore cover of adults killed in the burn. These new findings highlight that while early post-fire sampling is appropriate and useful, to accurately determine the response to fire in long-lived obligate-seeding species, much longer time scales of observation, on the order of decades, are required. This recent survey also indicates that burning should not be avoided as a management tool in supporting restoration of Morro manzanita, though stand age should be an important factor in considering the appropriate use of fire for a particular area. We would recommend against burning stands that are much younger than 40 yr; another decade or so of seed input may have substantially increased the viable seed and thus potential seedling recruits in the prescribed burn stand. At this time, since nearly all stands containing Morro manzanita are at least 74 yr old, with the exception of the 2 ha area described above, prescribed fire may be one of the most effective tools in ensuring the regeneration of new individuals in aging stands. This approach may not be feasible in some sites where there is close proximity to residential development, such as the Elfin Forest Preserve, even though these oldest stands may be experiencing declining soil seedbanks over time (Tyler and Odion 2022). In such sites, exposing seedbanks to fire-related

cues to stimulate germination, using small scale approaches such as burn boxes, or treating soil off-site could be suitable alternatives. Research to determine the efficacy and effectiveness of these restoration approaches are highly recommended.

Distribution and mapped occurrences.—Morro manzanita is restricted to a small portion of coastal area in and near Los Osos, San Luis Obispo County, California (Fig. 4 and 5). Its distribution is predominantly correlated with the Pleistocene eolian sand mapped as Baywood fine sand (Carpenter and Storie 1928; Soil Conservation Service 1984; Wiegers 2009), where there is no slope to moderate slope. Based on the distribution of Baywood fine sand in the Los Osos area (Fig. 3), the historic distribution of Morro manzanita is estimated to have covered 800 to 1,000 ha (USFWS 1994).

At the time of listing in 1994, it was estimated, based on aerial photos and surveys², that the geographic range of Morro manzanita was ~340 to 360 ha (Tyler and Odion 1996): one half consisted of small or low-density patches in and around developed areas, and one half consisted of more continuous or more dense stands with at least 50% cover. This represented a reduction of the species' geographic range to one third of its historical size due to removal of individuals or habitat elimination (Odion and Tyler 2002). By 2013 ~75% of historical habitat had been converted for residential use, resulting in highly fragmented populations (USFWS 2013).

While the geographic range in 1994 was reported to be ~350 ha, Tyler and Odion (1996) pointed out that this was an over-representation of the actual areal extent of the species because individuals are often present in low-density patches within a matrix of associated plant communities. In order to estimate the cover of Morro manzanita, Tyler and Odion (1996) recalculated to account for stands with a sparse cover of Morro manzanita having been equally weighted with stands with high cover. Using previously reported cover classes and estimated acreage of each (Mullany 1990; McGuire and Morey 1992), they estimated the area actually covered by Morro manzanita to be less than 162 ha (Tyler and Odion 1996).

Another source of information on the geographic range of the species is the California Natural Diversity Database (CDFW 2021), which includes spatial information associated with special status species and their recorded occurrences. For Morro manzanita there are six known occurrences, each with an assigned number by the California Natural Diversity Database (CDFW 2021). Their numbers are not in sequence. As knowledge of the species' distribution improved, some previously recognized occurrences have been combined with other occurrences, maintaining the criteria that separate occurrences are >0.4 km from any other occurrence. The assigned numbers for Morro manzanita occurrences remaining are 1, 4, 9, 18, 20 and 21 (Fig. 4). USFWS (2022) estimated the area of occupancy for the six occurrences, described below, using the California Natural Diversity Database (CDFW 2021) primarily from maps dated 1980 and 1990–1992.

For occurrence 1, CNDDDB (CDFW 2021) uses 28 ha as its area. Weislander and Schreiber (1939) give the location for paratype specimen (UC1334951/Ben Bolt 644/VTM14631) as “Valencia Peak”, collected 23 March 1936. They state the distribution of Morro manzanita as “sandy hills south of Morro Bay, 100–400 feet”, but Valencia Peak (Montaña de Oro State Park) is 1,345 ft/410 m elevation. Data in the pocket of the herbarium sheet give the following information: 1 mile east-northeast Valencia Peak; verbatim elevation as “400” ft; and habitat as woodland, north slope, and small type Monterey shale. California Natural Diversity Database (CDFW 2021) states “exact location unknown. mapped as best guess 1 air mile ENE of Valencia Peak”. USFWS biologist Emily Levin and California State Parks Environmental Scientist John Sayers visited Valencia Peak on 3 March and 30 March 2023, respectively. They

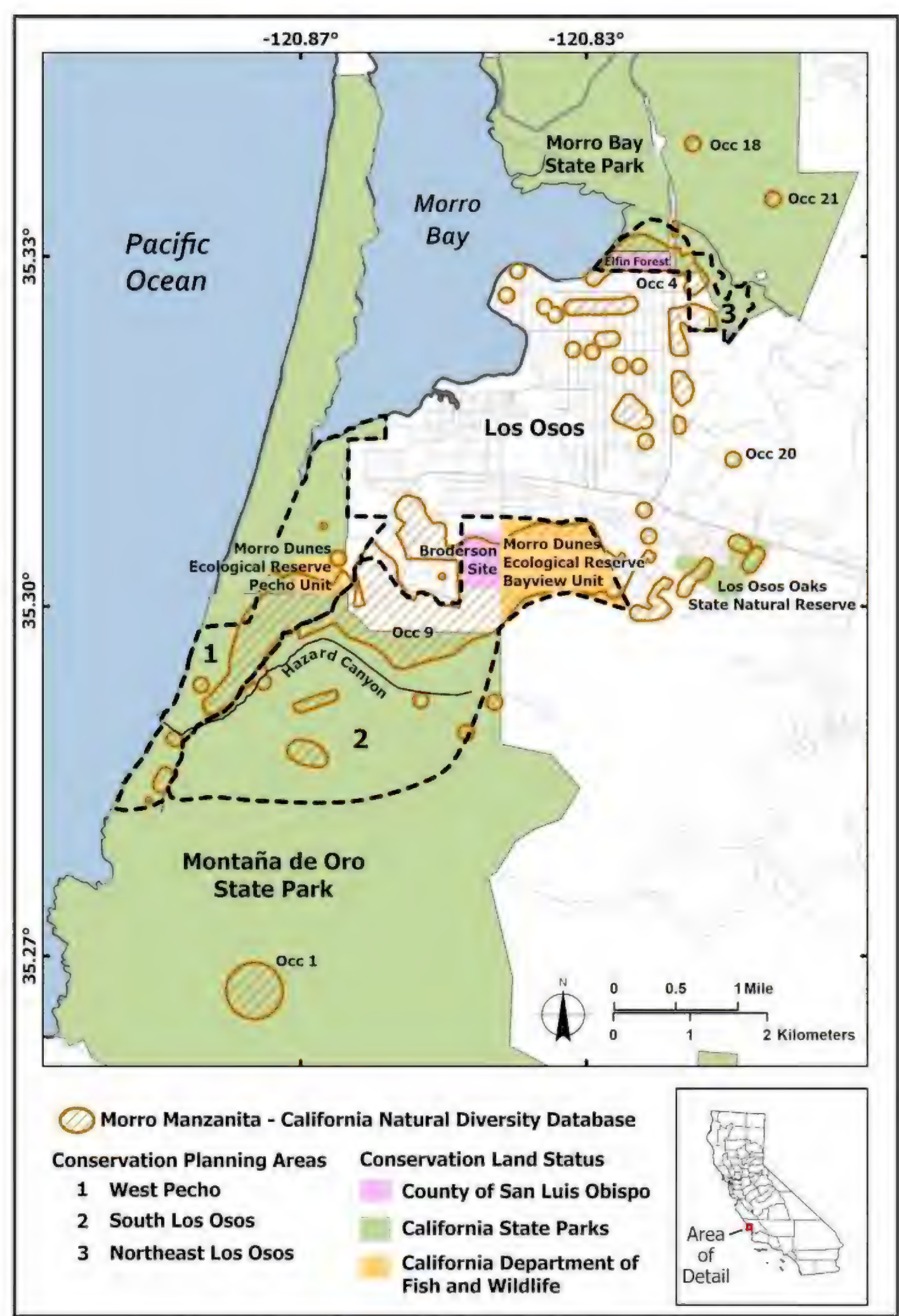


Fig. 4. Geographic distribution of Morro manzanita *Arctostaphylos morroensis* in west San Luis Obispo County, California, Conservation Planning Areas and Conservation Land Status (i.e., managing agency). We used GIS map layers for species occurrences in the California Natural Diversity Database (CDFW 2021), which are mostly from maps dated 1980 and 1990 to 1992. We include indications of new localities in Conservation Planning Area 2, and north and southmost individuals of Morro manzanita in Conservation Planning Area 1. Map credit: Mark Metevier, USFWS.



Fig. 5. Geographic distribution of Morro manzanita *Artocystaphys morrensis*, using GIS map layers of California Natural Diversity Database (CDFW 2021), excluding Valencia Peak, and underlain with 2023 Google Earth aerial image. Map credit: Mark Metevier, USFWS.

searched the area for Morro manzanita, finding none. In addition, we followed California Natural Diversity Database and used 1.6 km east-northeast Valencia Peak. We assigned the coordinates 35.26853, -120.85569 , 61 m north of Islay Creek in its valley at 109 m elevation, for this analysis. We hiked the Islay Creek Road to a point 391 m northeast of the coordinates at 77 m (253 ft) elevation, but we were prevented access from the road by dense chaparral and riparian vegetation, poison oak and rugged terrain. Bolt's stated location with Morro manzanita is possibly along Islay Creek Road, but we were unable to find it. More likely, "Valencia Peak" or 1 mile east-northeast Valencia Peak are erroneous data. It is possible that occurrence 1 was within the boundaries of what is now occurrence 9. Alternatively, the location itself was erroneous. In her MS thesis, Mullany (1990) describes Morro manzanita south of its typical range on "a ridge of Chamise shaly loam located approximately 0.25 mile south of Valencia Peak" (our emphasis added.) She also states that this "may be the area referred to in the original description as Valencia Peak. Other individuals of *A. morroensis* may have been overlooked on this ridgetop because of the difficulty of examining steep slopes and impenetrable chaparral. However, it is evident this is not part of the main range." Given Mullany's description, we recommend that the presence of Morro manzanita south of Valencia Peak should be investigated to determine if this occurrence remains valid.

Occurrence 4 is in north Los Osos. It is comprised of the Elfin Forest Preserve (14 ha), the adjacent part (21 ha) of Morro Bay State Park, and private land (69 ha), for a total estimated area of 104 ha (CDFW 2021). It is mapped as 16 polygons, mostly according to data from 1980 and 1990 to 1992. The majority of private land are residential parcels (Fig. 5); here some Morro manzanita are incorporated into residential landscaping (CDFW 2021) but they are likely substantially reduced in number and their ecological function is unknown.

Occurrence 9 is west of Pecho Valley Road, and south of the west end of Los Osos Valley Road extending to ridges south of Hazard Canyon. This occurrence includes multiple preserve properties as well as private land. It is mapped as 15 polygons, mostly according to map data from 1980 and 1990–1992, with an estimate of >152,200 plants. Its total area is 402 ha (CDFW 2021) and comprises the largest occurrence. We recorded the location of the southmost individual, which is within Montaña de Oro State Park, at 35.28145, -120.88440.

Occurrence 18 is at coordinates 35.34346, -120.8214, in Morro Bay State Park. It is 344 m east of South Bay Blvd and 74 m north of the Live Oak Trail, almost half way up the hill, 58 m elevation on the south facing slope, and 27 m east of the dead *Eucalyptus* trees. We observed ~12 individuals on 28 January 2023, with some apparent hybrids in the vicinity. We also observed here Oso manzanita *A. osoensis*. This is the northmost occurrence for Morro manzanita. The rocky, volcanic exposure of porphyritic dacite (igneous rock; Weigers 2009) is an unusual substrate for the species. The underlying substrate is mapped as Rock outcrop-Lithic Haploxerolls complex (Soil Conservation Service 1984). The only previous observation here was in 1989 (Mullany 1990) with one or a few individuals reported. CDFW (2021) estimates its area as 2 ha.

Occurrence 20 is at coordinates 35.31398, -120.81615, as mapped by Mullany (1990) at the eastern terminus of Freeman Lane in east Los Osos. Mullany (1990) reported a small group of plants (1 to a few individuals) in the 1980s growing with coast live oak *Quercus agrifolia* in dark, fine-textured soil. The dark, fine textured soil is a result of organic debris added to Baywood fine sand from the coast live oak. USFWS (2022) estimated the area of this occurrence as 2 ha. We searched for this occurrence of Morro manzanita on 28 January 2023, but without success. The occurrence is now likely extirpated. In the 1980s the terminus of Freeman Lane was 50 m northwest of the present-day terminus. House and facilities construction in 2005 probably removed the plants.

Occurrence 21 is at coordinates 35.33881, -120.81141, in Morro Bay State Park, 2.15 km east of South Bay Blvd., 68 m elevation. It is 19 m south of the Crespi Trail, on a south facing outcrop of shale (Nelson 2015). Soil Conservation Service (1984) mapped the underlying substrate as Rock outcrop-Lithic Haploxerolls complex. We visited the occurrence on 28 January 2023 and observed ~20 individuals, some with flowers and growing with Oso manzanita. The only previous observation of this occurrence was by Nelson (2015), who reported many plants. The area of this occurrence is estimated as 2 ha (CDFW 2021).

One new occurrence was identified in our November 2023 field surveys. Mullany (1990) wrote that “although *A. morroensis* is abundant along upper slopes of the Manzanita Trail, the steep slopes and similar appearance of the codominant *A. crustacea* precludes estimating cover there.” We confirmed the presence of Morro manzanita along the Manzanita Trail and East Boundary Trail in Montaña de Oro State Park at three localities not recorded in CNDDDB. These were: 35.29106, -120.85267; 35.28797, -120.84735; and 35.29085, -120.84406 (Fig. 4, 5). The first new locality with ~10 individuals is 0.37 km from occurrence 9, and thus would be included in this occurrence. The latter two (eastmost) comprise a new occurrence. The second new locality is 0.59 km from occurrence 9 (thus a new occurrence), and the third with one

individual is 0.66 km from occurrence 9. At the second locality, which was at the periphery of a rocky outcrop, we observed ~12 individuals of Morro manzanita and several brittle leaf manzanita *A. crustacea* subsp. *crustacea*. The underlying soil type for all three new localities is mapped as Santa Lucia shaly clay loam (Soil Conservation Service 1984). Data for these localities were submitted through online field survey forms to CNDDDB, and voucher specimens were deposited in the herbarium at UC Santa Barbara's Cheadle Center for Biodiversity and Ecological Restoration.

Summarizing the findings based on data for all occurrences of Morro manzanita from the California Natural Diversity Database (CDFW 2021) and our field surveys, we suspect that the location of occurrence 1 was based on erroneous data, and that occurrence 20 is no longer extant. Occurrences 9 and 4 represent at least 98% of this species' area of occupancy. The remaining three occurrences 18, 21 and our new occurrence are small but significant outlying stands at the edges of the species' range. Finally, the estimated total area of occupancy for this species using GIS data from the CNDDDB is 540 ha (this does not include our new occurrence data).

This estimate is much higher than the geographic range of ~350 ha cited by Tyler and Odion (1996) and others². We believe the CNDDDB derived figure, based primarily on maps dated 1980 and 1990 to 1992, is an overestimate for several reasons. Most significantly, the estimates for area of occupancy include land that has been converted to residential development. This is especially evident in occurrence 4 (Fig. 4) where many polygons mapped as Morro manzanita in north Los Osos are clearly lined by streets and dominated by houses (Fig. 5). This is also the case for some outlying polygons in occurrence 9 (Fig. 4) east of Morro Dunes Ecological Reserve Bayview Unit, and west of the Broderson Site (Fig. 4 and 5). In addition, occurrence 1 is likely to have been in error, meaning the 28 ha attributed to this occurrence is not correct. Instead, we suspect the records for this occurrence may be more accurately included in occurrence 9, or if it is present at a different location (south rather than north of Valencia Peak), and it is a much smaller area with likely only a few individuals, perhaps 1 ha total area. Thus, we suggest the previous estimate for the current geographic range of Morro manzanita as ~350 ha is more accurate than the 514 ha estimated in USFWS (2022) or 540 ha estimated above. However, there is clearly a need for updated information on the presence/absence of Morro manzanita within mapped polygons to better understand the current extent of this species, especially in the fragmented patches scattered across occurrence 4. At the same time, potential exists for additional outlying locations of Morro manzanita to be found. The species should be looked for at the periphery of the known geographic range. This includes east of South Bay Boulevard and north of Los Osos Valley Road, south and east of Shark Inlet (southmost point in Morro Bay) in Montaña de Oro State Park, and south of Hazard Canyon in Montaña de Oro State Park.

Current land management.—In the original species recovery plan, USFWS (1998) designated four “conservation planning areas”. These areas were identified and delineated to focus conservation efforts where multiple listed species would benefit and where recovery potential was high. The criteria were sites where: 1) the distributions of Morro manzanita, Morro shoulderband snail *Helminthoglypta walkeriana*, and Indian Knob mountainbalm *Eriodictyon altissimum* overlap or are contiguous with each other, 2) “natural habitats are relatively large and unfragmented by development”, and 3) “natural habitats are in public ownership or are adjacent to areas that are already secured and are to be managed for their biological diversity” (USFWS 1998). USFWS (2013) presented an amended version with three conservation planning areas (West Pecho, South Los Osos, Northeast Los Osos), which included the Morro manzanita's entire geographic range as known in 2013. USFWS (2022) maintained the amended version, with an updated geographic

Table 2. Properties with Morro manzanita *Arctostaphylos morroensis*. For each property we list the management agency, total area ha, Cover classes of Morro manzanita, size of the area covered with Morro manzanita, and the Conservation Planning Area associated with the property. Cover classes of Morro manzanita are from USFWS (2013): low = 1-25%, medium = 50-75%, high = 75-100%. Size of area with Morro manzanita was estimated using mostly GIS map layers of the California Natural Diversity Database (CDFW 2021), which are from maps dated 1980 and 1990-1992. Conservation Planning Areas are Northeast Los Osos, South Los Osos, and West Pecho (Fig. 4).

Property with Morro manzanita (MM)	Management agency	Total area (ha)	MM cover classes	Area with MM (ha)	Conservation Planning Area
Morro Bay State Park	CA State Parks	1,093	low	21	NE Los Osos
Elfin Forest Preserve	San Luis Obispo Co.	10	low	10	NE Los Osos
Elfin Forest Preserve	CA State Parks	4	low	4	NE Los Osos
Montaña de Oro State Park	CA State Parks	3,237	low, medium, high	139	S Los Osos and W Pecho
Morro Dunes Ecological Reserve Bayview Unit	CDFW	96	medium	83	S Los Osos
Morro Dunes Ecological Reserve Pecho Unit	CDFW	20	low	9	W Pecho
Broderson Site	San Luis Obispo Co.	32	medium	23	S Los Osos
Los Osos Oaks State Natural Reserve	CA State Parks	36	low	11	—
private land			low, high	237	—
			Total	537 ha	•

range of Morro manzanita. We show the current conservation planning areas and geographic range based on CDFW in 2021 (Fig. 4, Table 2).

Since the listing of Morro manzanita in 1994, California State Parks and CDFW have acquired substantial amounts of land in the vicinity of Morro Bay for conservation, including lands occupied by Morro manzanita. There are currently eight preserves that include significant cover of the species, and these are managed by three different agencies – California State Parks, California Department of Fish and Wildlife, and the County of San Luis Obispo (Table 2.) The preserves are distributed across the Conservation Planning Areas (Table 2). Approximately half of this area with Morro manzanita is managed by California State Parks, with their largest preserve (Montaña de Oro) included within the South Los Osos and West Pecho Conservation Areas (Fig. 4, Table 2).

Based on records from the CNDDDB there is a large area (total 237 ha) on private lands that is supporting Morro manzanita in low to high cover (Table 2). A small portion of this probably accurately describes some high cover stands that are in the South Los Osos Conservation Planning Area, north of Hazard Canyon and south-southwest of the Broderson Site. However, as described above in our discussion of the CNDDDB based maps of occurrences, we suggest this is a substantial overestimate because polygons in the developed portions of North Los Osos are unlikely to support even low cover of Morro manzanita at the present time. With the exception of the private lands south-southwest of the Broderson Site, the vast majority of properties supporting Morro manzanita are the named preserves (Table 2).

The first delisting criterion for Morro manzanita as stated in the recovery plan (USFWS 1998) is that 90% of existing core hectares supporting high (75 to 100%) and medium (25 to 75%) cover of Morro manzanita and 85 to 90% of low (1 to 24%) cover are secured from human-induced threats in the Conservation Planning Areas with no greater fragmentation. To

Table 3. Conservation Planning Areas (CPA) designated in the recovery plan for Morro manzanita (MM) by USFWS (1998) and referred to in the delisting criteria (USFWS 2022). For each CPA we list the total area ha, MM Morro manzanita cover in the CPA, area ha included in preserves, percent of CPA included in preserves, and list of preserves with ha that occur in the CPA. MM cover classes are from USFWS (2013): 1-25% = low, 50-75% = medium, and 75-100% = high. Areas were estimated using GIS map layers of the California Natural Diversity Database as reported in CDFW (2021). Conservation Planning Areas shown in Fig. 4.

Conservation Planning Area	Total area Ha	MM cover classes	Area ha in preserves	Percent of CPA in preserves	Preserves within the CPA and area (ha)
South Los Osos	243	medium, high	170	70%	Montaña de Oro State Park 64, Morro Dune Ecological Reserve Bayview Unit 83, Broderson Site 23
Northeast Los Osos	38	low	28	73%	Morro Bay State Park 14, Elfin Forest Preserve San Luis Obispo Co 10, Elfin Forest Preserve CA State Parks 4
West Pecho	84	low	81	97%	Montaña de Oro State Park 72, Morro Dune Ecological Reserve Pecho Unit 9
		Total:	279		•

assess progress toward that goal, we summarize the current status of hectares with various cover classes of Morro manzanita within the Conservation Planning Areas, and the preserves within each (Table 3). One Conservation Planning Area, South Los Osos, supports high and medium cover of Morro manzanita, and this is the largest unit at 243 ha. Seventy percent of the South Los Osos Conservation Area (170 ha), and thus 70% of hectares supporting high and medium cover of Morro manzanita is in three preserves: Montaña de Oro State Park, Morro Dunes Ecological Reserve Bayview Unit, and the Broderson Site (Table 3). The other two Conservation Planning Areas, Northeast Los Osos and West Pecho, both support Morro manzanita at low cover. These two planning areas combined total 122 ha, with 109 ha in preserves: Morro Bay State Park, Elfin Forest Preserve, Montaña de Oro State Park, and Morro Dunes Ecological Reserve Pecho Unit. Thus 89% of low cover Morro manzanita hectares is in preserves. Given these data, at this point in time the first delisting criterion appears not to have been met, as 70% rather than 90% of acreage with high and medium cover are protected. In addition, even within protected areas, Morro manzanita is not wholly secure from human-induced threats, as will be discussed below.

We note that the private land south of the Broderson Site and southwest of Cabrillo Estates supports high cover (75 to 100%) of Morro manzanita (Mullany 1990) (Fig. 4). This encompasses the most substantial portion of remaining unfragmented, intact hectares of Morro manzanita outside of preserves. Protecting these existing core stands from human-induced threats, such as through purchase and incorporation into existing preserves, would contribute greatly toward conservation of this species.

Threats.—Morro manzanita was listed as threatened under the U.S. Endangered Species Act in 1994 (USFWS 1994). The three main factors reported as contributing toward vulnerability of this species were residential development, competition from non-native plants, and stochastic events that negatively impact small, isolated populations (USFW 1994). Subsequent to the original listing, additional documents have addressed existing and potential new threats. A recovery plan was prepared in 1998 (USFWS 1998), and three 5-yr status reviews have been conducted, (USFWS 2008, 2013, 2022). In the first 5-yr review (USFWS 2008), status was reported to not be markedly different than at the time of listing. Two changes, one

positive and one negative, were noted at that time. One was the threat of habitat loss by development was substantially reduced due to transfer of private lands with the species to CDFW or California State Parks; however, there were no management plans directed toward Morro manzanita conservation. The other change noted was a new possible threat - altered fire regimes/fire management practices (USFWS 2008). In the second 5-yr review, the status was essentially the same as in the previous review, but with one new threat identified: climate change (USFWS 2013). All described threats are still on-going.

A continuing threat to the persistence of Morro manzanita and a primary factor in its listing is clearing of habitat for conversion to residential development. USFWS (1994) stated “the restricted range and narrow habitat requirements of *A. morroensis*, coupled with continuing alteration, destruction, and fragmentation of habitat, make it vulnerable to becoming endangered in the near future.” Although progress has been made in protecting maritime chaparral and coastal scrub with Morro manzanita through establishment of preserves (Table 2), threats by development on private lands remain. As described above, one of the largest remaining intact areas with high cover of Morro manzanita is on private land. Conversion of these stands to residences would be an irreversible loss of both Morro manzanita individuals and habitat capable of supporting this species. Such loss of habitat exacerbates the current negative impacts of fragmentation including reduced movement of pollinators and other associated species.

In addition, alteration of the habitat on private land adjacent to housing, due to current fire management practices, extends negative impacts into intact Morro manzanita stands. California’s new code section 51179 requires homeowners in areas at high risk of wildfire to maintain a defensible space around their homes, which is an area free of excess or dead vegetation. Ninety-five percent of occurrence 9 is an area designated very high risk, which is the most severe category. This includes the following housing estates in Los Osos: Vista Court, Cabrillo Estates, the Seascapes Place/Rodman Drive area, Bayview Heights, and Marguerite Drive mobile homes area. A homeowner must maintain a combustible-free zone of 1.5 m from the house, a lean/clean/green zone within 9 m feet of the house, and reduce potential fuel within 30 m feet of the house (Kerstein 2021; Calif. Dept. Forestry Fire Prevention 2023). In 2019, a fuel break (30 m wide) was constructed around the eastern edge of Cabrillo Estates, in which most vegetation was cleared and Morro manzanita severely pruned, removing the majority of shrub canopies and removing low branches contacting the ground. CA Department of Forestry and Fire Prevention is currently proposing to extend this fuel break to encompass the Seascapes Place/Rodman Drive area along Pecho Valley Road, and construct another fuel break from Cabrillo Estates eastward to the vicinity of Los Osos Oaks State Natural Reserve. Such intensive removal of manzanita biomass converts this former maritime chaparral habitat to open landscaping with denuded shrub-like specimens. The functional ecological value of these individual pruned manzanitas is unknown, though without doubt their reproductive output will be substantially reduced and habitat for associated wildlife will be altered. The original listing for Morro manzanita (USFWS 1994) acknowledged the past and future potential for such deleterious impacts stating that “in addition to direct removal of habitat, development has had secondary effects on quality of adjacent remaining habitat, such as fragmentation, deterioration of habitat due to increased recreational activity, and the introduction of non-native species.” Clearing for fuel breaks around residences that are adjacent to high cover Morro manzanita stands is another such secondary impact that poses a potential threat to the species. While maintaining defensible space is an important and valid public safety concern, it would be beneficial to explore alternatives to severe thinning of manzanitas beyond the 9 m requisite border, or to consider mitigation of impacts off-site.

It should also be noted that while establishment of preserves (Table 2) has been a critical step in reducing the extinction risk for Morro manzanita, even these protected areas are not

secured from human-induced threats. For example, targeted vandalism of Morro manzanita was reported in the Elfin Forest Preserve (P. Sarafian pers. comm. 2009). Invasive species, such as *Eucalyptus* discussed below, are abundant in Montaña de Oro State Park and other sites. To our knowledge, of the five relevant preserves, only the Broderson Site⁵ and Elfin Forest Preserve⁶ have final management plans, though plans that include specific strategies for the restoration and long-term conservation of Morro manzanita have not yet been implemented in any preserves or their respective Conservation Planning Areas. The management plan for the Los Osos Habitat Conservation Plan Preserve System⁷, which would incorporate the two units of the Morro Dunes Ecological Reserve, does include proposed strategies for habitat-based restoration in general, as well as specific measures for Morro manzanita protection and enhancement. These actions include removal of non-native species.

Competition from non-native, invasive plant species also remains a threat. Species described in USFWS (1994) included iceplant *Carpobrotus* sp., veldt grass *Ehrharta calcina*, and *Eucalyptus* spp. The latter is especially problematic. *Eucalyptus* plantations, as well as small stands, were planted in the early 1900's in Los Osos and within what is now Montaña de Oro State Park (Hook 1988). Based on the soils, Baywood fine sands, and adjacent vegetation, it is very likely that these were planted in sites formerly occupied by Morro manzanita (Mullany 1990). Where extensive *Eucalyptus* plantings abut dense Morro manzanita stands, few mature manzanitas remain under the *Eucalyptus* canopy and there is no regeneration there, perhaps due to competition for water or other biotic factors (Mullany 1990.) Also concerning is that expansion of *Eucalyptus* has been documented. In 1949 *Eucalyptus* covered 48.3 ha, by 1986 it had expanded to 73.5 ha (Bicknell 1990), and by 2021 it had further expanded to 141.6 ha (McFadden 2021). Finally, the extensive *Eucalyptus* plantations in Montaña de Oro State Park are highly flammable, and thus pose a wildfire risk. While Morro manzanita is adapted to fire, burning at increased frequencies (i.e., fire intervals under 40 yr) could lead to population declines. Dense cover of veldt grass poses a similar risk of altering fire regimes to the detriment of both Morro manzanita and other components of the maritime chaparral and dune scrub; similar impacts of invasive species have been documented across a variety of plant communities (Brooks et al. 2004). At the same time, the current *Eucalyptus* plantations offer opportunities for restoration and expansion of Morro manzanita. Removal of at least portions of these plantations followed by seeding or planting with Morro manzanita would allow for the re-establishment of this species into its former habitat. Potential locations for the initiation of such efforts would be the edges of *Eucalyptus* stands that have been recently thinned, such as along the East Cable Trail east of Pecho Valley Road in Montaña de Oro State Park (Fig. 6). Here, intact Morro manzanita stands persist at the outer edges of the plantations, and removal of 10 to 20 *Eucalyptus* could provide 100 m² of area to replant manzanitas, gradually reducing the area occupied by the plantations along the accessible periphery.

The third main threat identified in the original listing for Morro manzanita (USFWS 1994) was negative effects of stochastic events impacting small, isolated populations. Environmental stochastic events that could reduce abundance of Morro manzanita would include wildfires that occur at

⁵ SWCA Environmental Consultants. 2019. Habitat Management Plan for the Los Osos Wastewater Project, Los Osos, San Luis Obispo County, California. Prepared for County of San Luis Obispo. 112 pp.

⁶ Terra Verde Environmental Consulting. 2019. El Moro Elfin Forest Final Biological Assessment Report. Prepared for Los Osos-Morro Bay Chapter of Small Wilderness Preservation Area. 125 pp.

⁷ McGraw, J. 2020. Interim Adaptive Management and Monitoring Plan for the Los Osos Habitat Conservation Plan Preserve System. Prepared for County of San Luis Obispo, California Department of Fish and Wildlife, and U.S. Fish and Wildlife Service. 117 pp.



Fig. 6. Morro manzanita stands, indicated with arrows, at the edge of *Eucalyptus* plantation in Montaña de Oro State Park, south end of East Cable Trail. Photos by Peter Slaughter.

intervals too short for adequate seedbank stores to accumulate. Frequent fires have not been observed in the area, but increased spread of invasive grasses or flammable *Eucalyptus* species could alter the natural fire regime in this maritime climate. Demographic stochasticity refers to random fluctuations in reproduction and mortality, and in small populations these fluctuations can result in

reduced growth rates (e.g., Allee effect). Although further study is warranted, Tyler and Odion (2022) found that seed from Morro manzanita in the most isolated stand, the Elfin Forest Preserve, had significantly lower seed viability compared to other stands, 2% vs 4%, respectively. They hypothesized that low seed viability and high “infertility” (no evidence of embryo development) at the isolated stand may have been caused by inbreeding effects. Small, fragmented plant populations are susceptible to increased genetic drift and inbreeding (Sampson et al. 2016), which compromises plant reproduction (Aguilar et al. 2006). This may be particularly true for Morro manzanita, which is dependent on localized insect pollination (Tyler et al. 2023). Studies to describe the genetic diversity within and among patches would be useful in making decisions about the appropriateness of promoting gene flow in restoration plantings by using seed or cuttings from non-adjacent stands. Recent work by Huang et al. (2022) on the genomics of *A. glauca* may serve as a valuable reference for understanding genetic diversity in other manzanita species.

Climate change-driven drought may present a new threat to Morro manzanita as well as other endemic species with restricted distributions in the region’s maritime chaparral. Langridge (2018) provided a comprehensive assessment of how climate change will affect California’s Central Coast, including increased maximum/minimum temperatures, uncertainty in fog, slightly increased precipitation with substantially increased variability, increased extreme rainfall events, accelerated sea level rise, increased drought, and frequent and sometimes large wildfires. Mortality and stem die-off of several large Morro manzanitas in the Elfin Forest Preserve were observed since 2015 (over a period of extended drought), which may have been associated with the extremely low rainfall (P. Sarafian pers. comm. 2021). The tolerance of Morro manzanita to climate change is unknown, however, it is a habitat specialist in the coastal zone with marine fog. Morro manzanita cannot disperse to distant locations because it has a small geographic range and endemic soil requirements.

We highlight one new potential threat for Morro manzanita: the sudden oak death pathogen *Phytophthora ramorum*. Lee et al. (2019) and Frankel et al. (2020) reported the sudden oak death pathogen affecting multiple species of *Arctostaphylos*, including Morro manzanita in the botanic gardens of the University of California Santa Cruz in 2017 (M. Garbelotto pers. comm. 2022). In 35 yr, this disease had killed more than 50,000,000 trees in California and Oregon, primarily tanoak *Lithocarpus densiflorus* and coast live oak. Among eight species of *Arctostaphylos* tested for susceptibility, Morro manzanita was intermediate (Garbelotto et al. 2020). Although no infected plants of any species have been found in the wild in San Luis Obispo County, the nearest infections are 3 km north of the county line in Salmon Creek Canyon, southwest Monterey County (M. Garbelotto pers. comm. 2022), which is 72 km north of the nearest occurrence of Morro manzanita (occurrence 18). However, since 2019 the pathogen has been detected by polymerase chain reaction analysis in four streams in coastal San Luis Obispo County: Santa Rosa Creek (also known as Old Creek) 14.8 km north of occurrence 18 (6.6 km northwest of Cayucos); 34 km northwest of occurrence 18; San Simeon Creek, 38 km northwest of occurrence 18; and San Carpoforo Creek, 63 km northwest of occurrence 18. Despite intensive searches, no infected vegetation has been found in the watersheds (K. Corella pers. comm. 2023). Continued monitoring for the presence of this pathogen would be prudent since the potential consequences could be substantial.

Conclusions

We reviewed all available sources, reported new findings from recent field surveys, and evaluated the threats to Morro manzanita in 2023. Currently, the threats identified in the 1994 listing and subsequent 5-yr reviews are still on-going. Highest priority actions should include continued

efforts to preserve intact high-cover stands, as well as coordinated conservation and research to inform management in restoring and maintaining existing stands and facilitating recruitment. Based on the information presented here we provide the following recommendations:

- (1) Conserve and protect existing stands of Morro manzanita, with an emphasis on the largest remaining intact areas with high cover of Morro manzanita.
- (2) Encourage discussion with USFWS prior to fuel reduction impacting intact Morro manzanita stands in Los Osos, such as that conducted by CA Department of Forestry and Fire Prevention.
- (3) Conduct field surveys to improve the current distribution and abundance data for Morro manzanita. This should include verifying presence/absence of Morro manzanita in isolated patches mapped across Los Osos residential areas and others, as well as recording new locations. Submit these findings to the CNDDB.
- (4) Develop and implement site-specific management plans for Morro manzanita within preserves, including success criteria for evaluating effectiveness of management.
- (5) Develop protocols for long-term restoration success of Morro manzanita. Conduct research on viability and germination requirements of freshly collected manzanita seed to aid in restoration efforts.
- (6) Identify potential restoration sites across the Conservation Planning Areas both within protected areas to direct management efforts there, and within private land to be considered within potential habitat conservation plans. Investigate options for restoring connectivity between fragmented stands, including re-establishment of associated native plant and animal species.
- (7) Conduct research to describe the genetic diversity within and among existing stands/patches. If warranted by results of genetic diversity, when planting Morro manzanita for restoration, consider introducing some individuals, generated from seed or cuttings, from non-adjacent stands to enhance gene flow and genetic diversity, especially for isolated stands.
- (8) Remove *Eucalyptus* and re-establish Morro manzanita where feasible in the southwest part of the range including sites along Pecho Valley Road. Potential *Eucalyptus* selected for removal would exclude those individuals identified as Monarch butterfly roost sites or important wind breaks.
- (9) Coordinate and share information between agencies, researchers and citizen groups including the San Luis Obispo Chapter of the California Native Plant Society, and Friends of El Moro Elfin Forest, who are involved with outreach and conservation of Morro manzanita.
- (10) Continue studies of the relationship of Morro manzanita with fire.
- (11) Conduct prescribed burns of vegetation in Los Osos to reduce the risk of wildfire. This would also benefit Morro manzanita by stimulating germination and establishment of new seedlings.
- (12) Conduct modeling to anticipate effects of climate change on distribution and abundance of Morro manzanita, including changes in temperature, precipitation, amount and extent of marine fog layer, and sea level rise.
- (13) Collect seeds of Morro manzanita for conservation seed banking.
- (14) Introduce Morro manzanita (with representative genetic diversity) into living collections at several botanic gardens.

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Exploring Commensalism Between Rock Wrasse (*Halichoeres semicinctus*) and Round Stingrays (*Urobatis halleri*) in Southern California

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Abstract.—Positive interactions are underrepresented in marine ecology but have a substantial impact on biodiversity and ecosystem stability. Here, we showcase a previously undescribed commensal relationship between two temperate rocky reef fishes in sandy bottom habitats: the rock wrasse (*Halichoeres semicinctus*) and round stingray (*Urobatis halleri*). Using snorkel surveys in Big Fisherman's Cove on Santa Catalina Island, we showed that rock wrasse abundances were positively associated with the presence of round stingrays and that round stingrays significantly altered rock wrasse behavior. Specifically, rock wrasse within a 1 m radius of a feeding round stingray spent approximately 40% and 35% more time feeding compared to rock wrasse in proximity of a resting round stingray or a sandy bottom control, respectively. The positive effect of feeding round stingrays on rock wrasse feeding behavior is in response to stingrays disturbing sand as they eat, uncovering small invertebrates for the wrasses to prey on. As round stingrays are one of the most common fishes in southern California, they may impact the fitness of rock wrasses.

Interspecific interactions can shape food webs, habitats, and structure marine communities (May 1972; Wootton and Emmerson 2005). Historically, ecological studies have focused primarily on antagonistic interspecific relationships such as predation, parasitism, and competition (Mathis and Bronstein 2020). Positive interactions such as commensalism (or facilitation) and mutualism, are generally overlooked (Bertness and Callaway 1994; Bruno et al. 2003). Although, studies on mutualisms, an interspecific interaction in which both species benefit, are now more prominent in the literature (Mathis and Bronstein 2020), possibly because positive interactions are favored by natural selection (Bronstein 1994; Johnson et al. 2021) or because there is evidence for positive interactions increasing habitat stability (Stachowicz 2001). Between the two types of positive interactions, commensalism—the relationship between two species in which one benefits while the other remains unaffected—is still comparatively underrepresented relative to mutualisms, especially within marine research (Mathis and Bronstein 2020). Notably, commensal relationships are more represented in terrestrial (Dickman 1992) and freshwater studies (Silknetter et al. 2020). Here, we showcase a previously unknown commensal relationship in temperate marine fishes and highlight the general importance of understanding commensal relationships in marine ecosystems.

Within the marine realm fishes are a focal point for better understanding the importance of positive interactions. In the Labridae, for example, mutualisms between the cleaner wrasse (*Labroides dimidiatus*) and other resident reef fishes are heavily researched and are shown to facilitate biodiversity (Caves 2021; Waldie et al. 2011). *Labroides dimidiatus* was recorded

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consuming up to 1200 ectoparasites in a single day, allowing them to fulfill a vital function within coral reef ecosystems, influencing the diversity, abundance, size, and recruitment of reef fish via parasite removal (Caves 2021). An 8.5 yr removal of *L. dimidiatus* revealed a significant decline in species richness on the Great Barrier Reef along with changes to resident community structure, recruitment success, and size frequency distribution of other reef fish like the damselfishes *Pomacentrus moluccensis* and *P. amboinensis* (Waldie et al. 2011). In another example, a carnivorous wrasse (*Thalassoma duperry*) formed a mutualistic relationship with the green sea turtle (*Chelonia mydas*) in which the wrasse removed ectoparasitic barnacles from the skin of the turtle (Losey et al. 1994). The sea turtles provided *T. duperry* with food while the removal of ectoparasites by the wrasse seemingly increased the health of *C. mydas* by reducing drag and lowering the chance of infection (Losey et al. 1994). While many wrasses within labrids are the focus for research on interspecific relationships in marine environments, rock wrasse (*H. semicinctus*) differ as they are predominantly foragers rather than cleaners (Hobson and Chess 1986).

Rock wrasse live in temperate rocky reefs, sandy bottoms, and kelp forests from the Gulf of California to Point Conception, California (Miller and Lea 1972). Rock wrasse are opportunistic feeders often found picking off small invertebrates from macroalgae and in disturbed sediments on sandy bottoms (Hobson and Chess 1986). The geographic range of rock wrasse also overlaps with another common temperate fish species, the round stingray (*Urobatis halleri*) (Valadez-González et al. 2001), which also feeds on invertebrates on sandy bottoms (Valadez-González et al. 2001). The round stingray utilizes its pectoral fins to disturb sediment on the sandy bottom floor to uncover prey such as stomatopods, decapods, and amphipods that compose a large portion of their diet (Valadez-González et al. 2001). This feeding practice may result in the displacement of small burrowing invertebrates such as nematodes, porifera, mollusks, echinoderms, and crustaceans from their habitats into the water column, making them readily available prey for the rock wrasse. These sand-dwelling invertebrates are common prey items for rock wrasse (Johnson et al. 1994). We hypothesized that there is a commensal relationship between round stingrays and rock wrasse, where stingrays facilitate rock wrasse by making prey easily available to them. By exploring this interspecific relationship, we plan to identify the dynamics of a potential commensal relationship between two fish species.

Materials and Methods

This study consisted of a combination of behavioral and abundance surveys of rock wrasse and round stingrays during the months of October and November 2022 at Big Fisherman's Cove, Santa Catalina Island, California, USA (33° 26' 39.1" N, 118° 29' 06.2" W; Fig. 1). Big Fisherman's Cove is located within the Blue Cavern Onshore State Marine Conservation Area which is a no take zone. Rock wrasse and round stingrays are both common fishes at this site (Pondella and Allen 2000; Froeschke et al. 2006). All observations were made at depths ranging from 3 to 5 m and during daylight between the hours of 08:30 and 17:00 as rock wrasse are a diurnal species that bury themselves in the sand at night (Hobson and Chess 1986).

We tested the hypothesis that rock wrasse actively feed more in the presence of feeding round stingrays relative to resting round stingrays or a no stingray control using timed snorkel surveys. Timed snorkel surveys are a commonly used method in fish behavior studies (Lawson et al. 2011; Weaver et al. 2014). Stingray resting behavior was defined as a stingray lying motionless within or on top of the sand during the entire timed survey period. Feeding behaviors were defined as the round stingray disturbing the sand to consume prey. All control

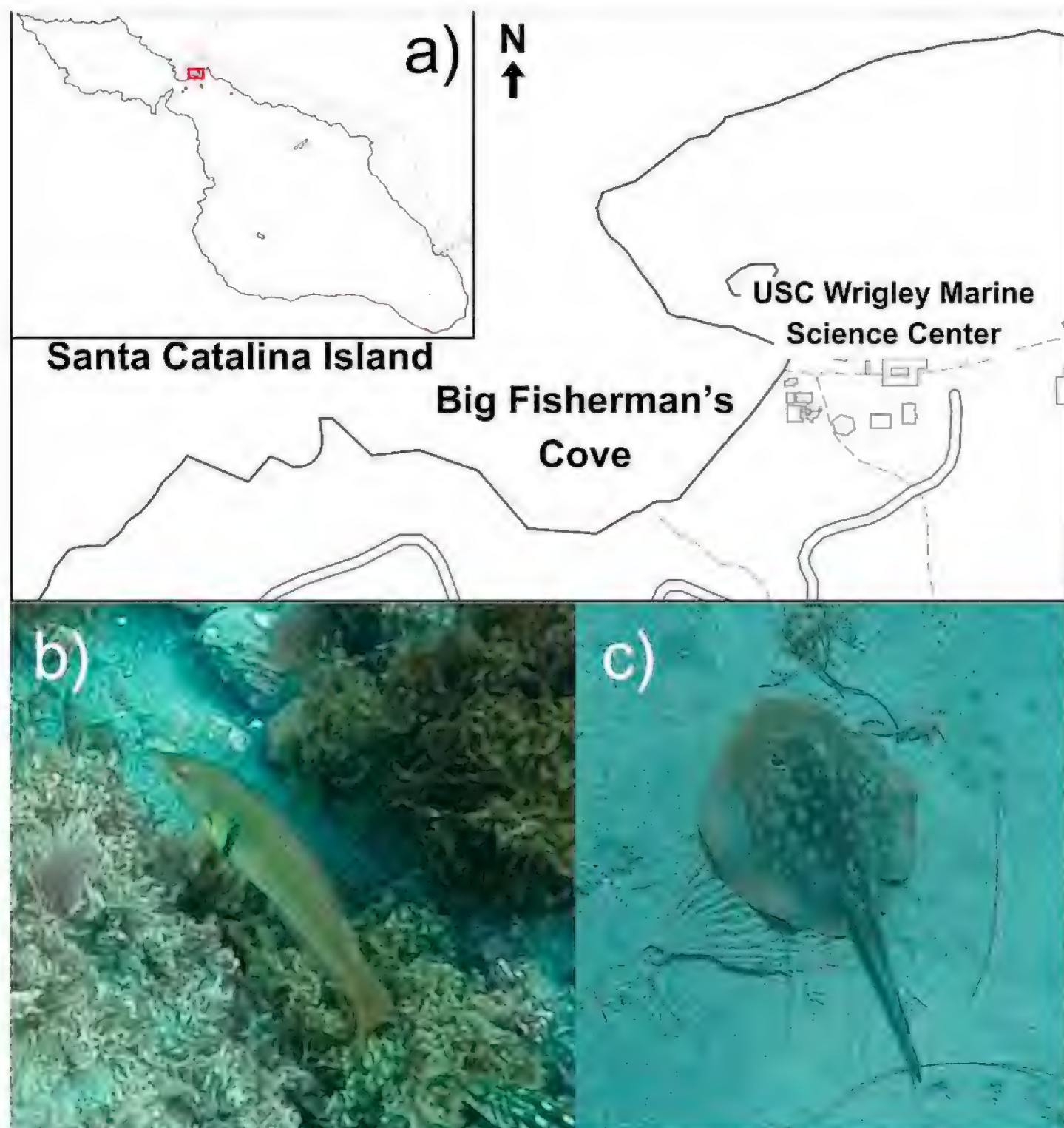


Fig. 1. (a) Geographic location of the study site at Big Fisherman's Cove, Santa Catalina Island. Insets (b) and (c) are photos of the rock wrasse and round stingray, respectively.

samples were taken at locations along the sandy bottom where no stingrays were present within 2 m of a rock wrasse. We haphazardly selected 85 rock wrasse individuals to observe for one minute each: 27 were within 1 m of a resting round stingray, 27 were in the control group with no round stingray present, and 31 were within 1 m of a feeding round stingray. The rock wrasse's behaviors were categorized as either traveling, chasing, or feeding and each behavior was measured to the nearest second using a waterproof stopwatch (Freeman and Grossman 1992). Traveling behavior consisted of the rock wrasse moving through the water column, but not feeding. Chasing behavior consisted of the observed rock wrasse either chasing another fish away or being chased by a fish. Lastly, feeding behavior consisted of foraging within macroalgae, picking through sediment, or snatching prey out of the water column. It is important to note that rock wrasse measurements in the presence of stingrays may have been near more than one stingray, as stingrays tend to feed in groups. In addition to rock wrasse behavior, we measured the instantaneous

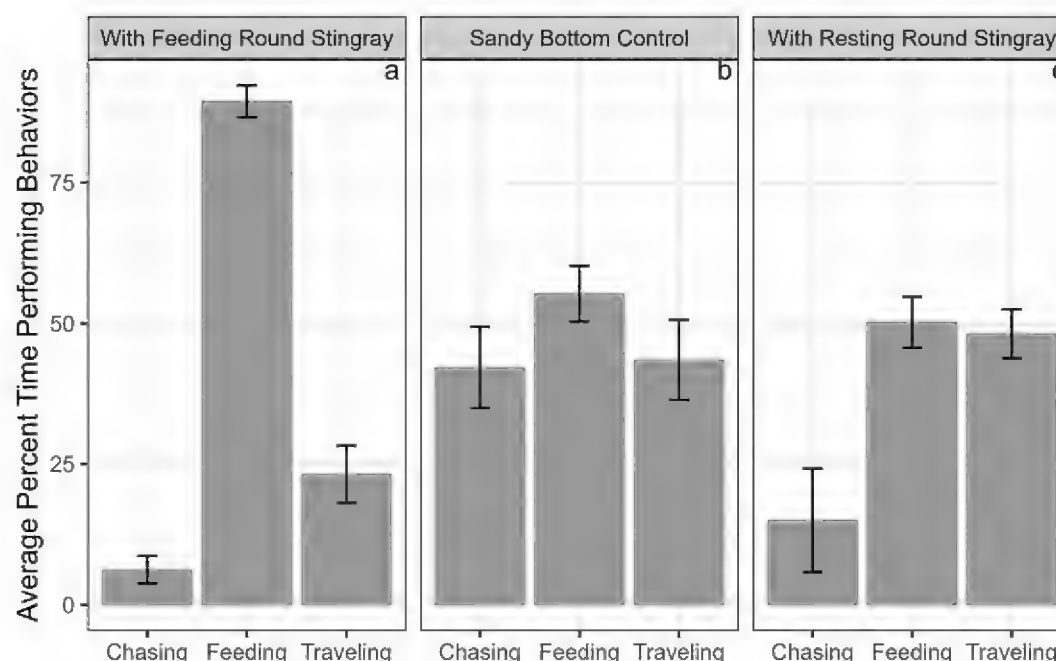


Fig. 2. Mean percent time $\pm$ standard error spent by rock wrasse chasing, feeding, and traveling when within one-meter radius of a (a) feeding round stingray ($n = 31$), (b) sandy bottom control ($n = 27$), and (c) resting round stingray ($n = 27$).

abundance of rock wrasse associated with 75 resting and 75 feeding round stingrays. A rock wrasse was labeled as associated with the stingray if it was within a 1m radius of the focal stingray. Rock wrasse abundance surveys were conducted using a zig-zag pattern across the sandy bottom to identify round stingrays and count the abundance of rock wrasse within 10sec of locating a stingray.

We tested the effect of stingray behavior (resting, feeding, no stingray control) on wrasse behavior (feeding, chasing, traveling) with a two-way ANOVA. The percentage of time within an activity during a one-minute sample was the response variable and stingray and wrasse behavior categories were included as interacting predictor variables. A Tukey HSD test was utilized to differentiate significant differences between the wrasse behaviors across each group. Assumptions for the two-way ANOVA were assessed by visualizing the distribution of the residuals using the *performance* package in R (Lüdtke et al. 2021). To analyze differences in rock wrasse abundance across resting versus feeding round stingrays, we used a generalized linear model with a Poisson distribution to account for non-normality within the data. All analyses were conducted using the program R (Version 4.2.1).

Results

The behavior of the round stingray significantly altered the behavior of the rock wrasse ($F_{4,155} = 17.52$, $p < 0.0001$; Fig. 2), with the greatest impact on feeding. Overall, the rock wrasse spent the majority of the time feeding when in the presence of a feeding round stingray ($89.41\% \pm 2.82$, $p < 0.0001$) and spent 39.2% and 34.1% more time feeding near a feeding round stingray compared to a resting round stingray ($p < 0.0001$) and a sandy bottom control ($p < 0.0001$), respectively. Rock wrasse near feeding round stingrays spent significantly less time on average chasing ($6.25\% \pm 2.43$) compared to rock wrasse with no round stingray present ($42.14\% \pm 7.24$; $p = 0.01$; Fig. 2). Rock wrasse associated with feeding stingrays also spent less time traveling ($23.19\% \pm 5.06$) than rock wrasse with a resting round stingray ($48.15\% \pm 4.31$; $p = 0.04$) or a sandy bottom control ($43.52\% \pm 7.11$; Fig. 2). The abundance of rock wrasse was significantly higher near feeding rays (1.07 ± 0.06 , $n = 75$) than resting rays (0.00 ± 0.00 , $n = 75$) during the snorkel count surveys ($X^2_{(1)} = 28.42$, $p < 0.0001$). Notably, no rock wrasse

were present near resting stingrays during the instantaneous surveys whereas the highest number of wrasses documented near a feeding round stingray was three.

Discussion

This study provides evidence for a commensal relationship between the rock wrasse and the round stingray in sandy bottom habitats based on both behavioral and abundance data. The significant increase in rock wrasse time spent feeding and their abundance in the presence of a feeding round stingray indicates that rock wrasse individuals benefit from stingrays. Notably, the strength of this relationship will change seasonally as stingrays migrate to warmer waters in the winter throughout southern California (Allen et al. 2002; Hoisington and Lowe 2005; Vaudo and Lowe 2006). Further, as rock wrasse are opportunistic feeders and are not reliant on stingrays for sustenance (Hobson and Chess 1986), it is likely that the rock wrasse and round stingrays exhibit a facultative commensal relationship. Considering the seasonal variability in round stingray abundance and the dynamic nature of the rock wrasse's ecological interactions as an opportunistic feeder, more research is required to identify and understand the range of biotic relationships taking place in this system. Because many temperate fish species disturb sediment while searching for prey, rock wrasse may have many commensal relationships with other fishes and invertebrates. Specifically, we noted rock wrasse feeding around California sheephead (*Bodianus pulcher*), halfmoon (*Medialuna californiensis*), opaleye (*Girella nigricans*), garibaldi (*Hypsypops rubicundus*), sargo (*Anisotremus davidsonii*), shovelnose guitarfish (*Rhinobatos productus*), and the California two-spot octopus (*Octopus bimaculoides*) at Santa Catalina Island. The relative importance of these different bioturbators to rock wrasse should be further explored.

Rock wrasse may experience increased fitness due to the feeding behaviors of round stingrays and other bioturbators by making the rock wrasse's prey items more easily accessible. When rock wrasse feed along sandy bottoms, they use their anterior canines to meticulously search for and pick out prey items within the sand (Hobson and Chess 1986). By utilizing the feeding round stingrays, rock wrasse can single out prey in the sand clouds, which likely reduces search time and energy expenditure for finding food. Foraging fish are faced with an important risk trade-off when searching for food availability while avoiding predation (Milinski 1992). Fish, such as the bluegill sunfish (*Lepomis macrochirus*), have been shown to consciously weigh profitability of feeding against predation (Werner et al. 1983). To minimize predation risk, foraging fish often seek complex habitats for safety, decrease foraging behavior, and reduce their feeding range (Hammerschlag et al. 2010; Holbrook and Schmitt 1988; Milinski 1992; Werner et al. 1983). However, rock wrasse in this study are utilizing a high-risk feeding area due to the openness of the sandy bottom habitat in which round stingrays are feeding. This increased predation risk of foraging in an open area may be counteracted by the reduction in foraging time as a result of the round stingray increasing food availability. Increased food availability has been shown to not only affect growth rate and survival in fishes (Werner et al. 1983), but also improve reproductive success in coral reef damselfish (*Acanthochromis polyacanthus*) (Donelson et al. 2010). Further research is needed to expand our understanding of the individual and ecological impacts this species interaction may have on rock wrasse fitness.

While we did not specifically test the effect of rock wrasse on round stingrays, the round stingrays appeared indifferent to the presence of rock wrasse hovering above them, further suggesting that this is a commensal relationship as only one species benefitted from the interaction (Mathis and Bronstein 2020). Two types of commensal relationships have been identified with respect to the non-benefiting species: no-effects commensalism or balanced-cost-and-benefits

commensalism (Mathis and Bronstein 2020). No-effects commensalism occurs when one of the species is completely unaffected from the interaction, while balanced-cost-and-benefits commensalism occurs when the cost and benefit of the interaction equal each other resulting in a net null effect. We propose that rock wrasse and round stingrays exhibit a no-effects commensalism as we never saw any behavioral changes in the stingray while in the presence of a rock wrasse, though this should be explicitly tested in the future. No effects commensalisms have been shown in other marine fishes such as the Carapini fish (*Carapus boraborensis* and *C. homei*) with their holothurian host (*Bohadschia argus*) during metamorphosis (Parmentier and Das 2004; Mathis and Bronstein 2020) and the juvenile jack mackerel (*Trachurus japonicus*) observed utilizing the bell cavity and tentacles of moon jellyfish (*Aurelia aurita*) for food collection and predator avoidance (Masuda et al. 2008).

Commensalism has been generally overlooked in marine ecology despite playing a major role in community dynamics (Mougi 2016; Bertness and Callaway 1994). For example, in Costa Rica, commensalism between large marine predators and fish-eating birds resulted in an enriched biodiversity at the lagoon where the commensalism was present compared to a lagoon that the interaction was absent (Deffebach et al. 2012). Indeed, further investigation into species interactions has changed our understanding of “textbook” examples of symbiotic relationships. It remains crucial to gain a better understanding of commensalisms as this species interaction has been shown to increase ecosystem stability and can positively influence recruitment, species distribution, and succession (Bertness and Callaway 1994; Mougi 2016).

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CONTENTS

Nebalia holothurophila, a New Species of the Leptostraca (Crustacea, Malacostraca)
Possibly Commensal with the Sea Cucumber, *Apostichopus parvimensis* (H. L.
Clark, 1913), in Southern California. Joel W. Martin, Jerry Jacobs, Todd A.
Haney, and Adam Wall ----- 1

Phylogeography of the Pacific Sardine, *Sardinops sagax*, across its Northeastern
Pacific Range. Ella S. Adams and Matthew T. Craig ----- 10

Ecology and Conservation Status of Morro Manzanita *Arctostaphylos morroensis*:
A Threatened Plant Endemic to Los Osos, San Luis Obispo County,
California. Claudia M. Tyler and Christopher P. Kofron ----- 25

Exploring Commensalism Between Rock Wrasse (*Halichoeres semicinctus*) and
Round Stingrays (*Urobatis halleri*) in Southern California. Bailey Bonham
and Nyssa J. Silbiger ----- 53